Comparative phytochemical analysis and *in vitro* antimicrobial activities of the cyanobacterium *Spirulina platensis* and the green alga *Chlorella pyrenoidosa*: potential application of bioactive components as an alternative to infectious diseases

Analyse phytochimique comparative et étude in vitro des activités antimicrobiennes de la cyanobactérie Spirulina platensis et de l'algue verte Chlorella pyrenoidosa : application potentielle des composants bioactifs comme alternative aux maladies infectieuses

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Abstract. Microalgae exhibit a huge genetic diversity. Cyanobacteria (blue-green algae) are rich sources of structurally novel and biologically active metabolites. Recent studies indicated the presence of some bioactive compounds in the blue green algae which are shown to exhibit anticancer, antimicrobial, antifungal or anti-inflammatory and other pharmacological activities. The increased use of antibiotics in the treatment of infectious diseases has led to the emergence of Multi-resistant forms of pathogenic bacteria and the imbalance of the microbiota. This work was conducted to evaluate *in vitro* the antibacterial and antifungal activities of two microorganisms *Spirulina platensis* and *Chlorella pyrenoidosa* against pathogenic bacteria and fungi using Agar well diffusion method and Paper disc diffusion method. The peroformed preliminary phytochemical analysis of this two dried algal samples reveals that these assays withhold some of the valuable bioactive compounds with the highest percentages of the total phenolic and total flavonoid contents in *Chlorella*. In present screening methanolic extract from *Spirulina* exhibited widespread spectrum of antimicrobial activities (43 ± 4.24 mm) and minimum inhibitory concentrations (MIC) 128 ± 0.71 µg/ml. Organic extracts from *Chlorella* shown that they excreted a broad spectrum of antimicrobial substances against Gram negative bacteria. However, organic extracts from *Spirulina* were appeared to be the most promising antibacterial activity against Gram positive bacteria. The result of chemical analyses showed that *Chlorella* recorded the highest percentages of the total phenolic, total flavonoid contents and chlorophyll. The algal extracts are potentially prolific sources of highly bioactive secondary metabolites that might lead in the development of new pharmaceutical agents for the treatment of bacterial infections.

Keywords: Microalgae, antibiogram, algal extract, phycochemical composition, Antibiotics, MIC.

Résumé. Les microalgues présentent une diversité génétique énorme. Les cyanobactéries (algues bleu - vert) sont des sources des nouvelles structures et des métabolites biologiquement actives. Des études récentes ont montré la présence de certains composés bioactifs dans les algues bleu-vert qui sont doués des activités anticancéreuses, antimicrobiennes, antifongiques, anti-inflammatoires et autres activités pharmacologiques. L'utilisation accrue des antibiotiques dans le traitement des maladies infectieuses a conduit à l'émergence de formes multi-résistantes de bactéries pathogènes et le déséquilibre du microbiote. Ce travail a été mené pour évaluer *in vitro* l'effet antimicrobien des extraits organiques des deux microorganismes *Spirulina platensis* et *Chlorella pyrenoidosa* à l'égard des bactéries pathogènes résistantes aux antibiotiques par la méthode de diffusion sur gélose en puits et des disques. Les analyses phytochimiques préliminaires de la poudre de ces deux algues révèlent la présence de certains composés bioactifs avec le pourcentage le plus élevé de la teneur en composés phénoliques et des flavonoïdes totaux dans la Chlorelle. Dans le présent criblage l'extrait méthanolique de la spiruline présente le spectre d'activité le plus important (43 ± 4.24 mm) et un IMC de 128 ± 0.71 µg/ml. Les extraits organiques de la Chlorelle montrent le plus grand spectre d'activités antimicrobiennes contre les bactéries à Gram négatif. Par contre, les extraits de la spiruline semblent être doués d'activités antibactériennes les plus prometteuses contre les bactéries à Gram positif. Le résultat des analyses chimiques ont montré que la *Chlorella* présente les plus forts pourcentages en composés phénoliques, en flavonoïdes et en chlorophylle. Les extraits d'algues sont des sources potentielles de métabolites secondaires bioactives qui pourraient conduire à l'élaboration de nouveaux agents pharmaceutiques pour le traitement des infections bactériennes.

Mots-clés : Microalgue, antibiogramme, extrait d'algue, composition phytochimique, *antibiotique*, CMI.

INTRODUCTION

Microbial infections are one of the prominent causes of death and health problems, physical disabilities throughout the world. Infections of the intestinal tract including Diarrhea and gastroenteritis are common, affecting people of all ages.

The increasing interest in the use of alternative therapies is the result of the development of antibiotic resistance in bacteria becoming a major problem and because people are experiencing the sometimes-severe side effects of many be sufficient to give rise to an aversion to all synthetic drugs (Molan 1999). There is an urgent need for development of alternative treatment therapies from various natural sources including microalgae (Mundt *et al*. 2001, Safonova & Reisser 2005, Ghasemi *et al*. 2007, Prakash *et al*. 2011) against infectious diseases. Algae are now drawing a greater interest following the increase in demand for biodiversity in the screening programs seeking therapeutic drugs from natural products. Microalgae exhibit a notable biodiversity; they can in fact be found as individual cells, colonies or extended filaments. Some researchers have envisioned the enormous possibilities of algae and microalgae as potential source of bioactive compounds (Borowitzka *et al*. 1988, Goud *et al*. 2007, Kaushik *et al*. 2008). The secondary metabolites present in algae are in favor of organizing a

numerous biological defense systems (Findlay *et al*. 1984, De lara Isassi *et al*. 2000) particularly, some microalgae have been studied as a potential natural source of different functional compounds (Herrero *et al*. 2006, Rodri'guez-Meizoso *et al*. 2008, Hristo Najdenski *et al*. 2013, Entesar Ahmed 2016).

They have been used in traditional medicine for a long time and as some algae also proved to have bacteriostastic, bactericidal, antifungal, antiviral and antitumor activities (Justo *et al*. 2001).

Spirulina and *Chlorella* are known to produce a wide range of secondary metabolites with various biological actions (Kaushik *et al*. 2009, Ghasemi *et al*. 2007). The microalgae can also be exploited as a potential source as food, feed and fuel (Akhtara *et al*. 2012).

Cyanobacteria called also blue-green-algae are one of the most diverse groups of Gram-negative photosynthetic prokaryotes (Muthulakshmi *et al*. 2012), widely distributed throughout the world. *Spirulina* (Arthrospira) is referred to free-floating filamentous microalgae with spiral characteristics of its filaments (Sapp 2005, Komárek *et al*. 2009).

The United Nations world food conference declared *Spirulina* as "The best for tomorrow" (Kapoor*et al*. 1993). Its protein's content varies between 50 and 70 % dry weight. Moreover, the best sources of vegetable proteins achieve only half these levels; for example, soya flour contains "Only" 35 % crude proteins.

Chlorella is a single celled green alga (Beijernick 1890) with spherical cells in shape tending to aggregate into colony; yellowish green, 4 to 8 µm in diameter and is without flagella. *Chlorella* contains the green photosynthetic pigments chlorophyll-a and b- in its chloroplast often with one pyrenoid, situated in the middle. *Chlorella* is a nutrient-dense super food that contains high level of proteins (50 to 70 % of dry matter) which 18 amino acids (including all the essential amino acids), lipid, vitamins and minerals (Phang 1992).

Several studies indicate the presence of some bioactive compounds in the freshwater blue-green algae which are shown to inhibit growth of several representatives of Gram-positive and Gram-negative bacteria (Vepritskii 1991), exhibit anticancer, antimicrobial, antifungal or anti-inflammatory (Kailash *et al*. 2010), enzyme inhibiting, immunostimulant, cytotoxic and antiplasmodial activities (Ghasemi *et al*. 2004). Pratt *et al*. (1944) were the first which have isolated an antibacterial substance from *Chlorella.* Very little experimental studies carried out under *Chlorella* have demonstrated its antitumor effect, cancer chemoprevention properties, anti-inflammatory, antioxidant and antimicrobial activities (Wang *et al*. 2010, Guzmán *et al*. 2001, Vijayavel *et al*. 2007, Makridis *et al*. 2006).

The aim of the present study is to evaluate the phytochemicals and antibacterial properties of the petroleum ether, hexane and acetone, dichloromethalonic and methanolic extracts of the two dried microorganisms *Spirulina platensis* and *Chlorella pyrenoidosa* against eight bacterial strains of reference and two fungal strains.

MATERIAL AND METHODS

Microalgae samples and culture conditions

The microalgae *Spirulina* and *Chlorella* are pure cultures dried and sold as dietary supplements in Tchad and France, respectively. The blue-green alga, *S. platensis* pure culture sample were cultured in 500 ml of Zarrouk medium as was reported by Zarrouk (1966) and Raoof *et al.* (2006).

Then de culture was maintained under controlled conditions at room temperature 25 ± 2 °C and continuous light of 7.5 $\mu.mol.m^{-2}.s^{-1}$ provided by white fluorescent tubes. The pure culture sample of *Chlorella* was kept under fluorescent light (20 $\mu.mol.m^{-2}.s^{-1}$) and a photoperiod of 16 h light 8 h dark at 24 ± 1 °C in Bold's Basal Medium (BBM) (Bischoff *et al*. 1963, Bold, 1949). Pure cultures prior to the stationary phase of growth (5-6 days) were harvested and filtered under vacuum using filter membrane (0.45 µm) and washed several times with distilled water. Then, the algae cells were dried at 60 °C for 30 min.

Tested microorganisms

The microorganisms used in antibacterial assays were supplied by Microbiology laboratory, Institute Pasteur, Alger, Algeria. The species employed include pathogenic Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC 10876, *Bacillus subtilus* ATCC 6633, *and Bacillus subtilus* ATCC 9372) and Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella* sp ATCC 4352, *Salmonella typhimurium* ATCC 14028 and *Pseudomonas aeroginosa* ATCC 27853) and two fungi *Candida albicans* ATCC 10231 and *Aspergillus* sp. ATCC 16404. All bacterial strains were stored at - 20 °C in nutrient broth medium supplemented with 30% of glycerol.

For MIC experiments and for agar diffusion test, bacterial inoculum was prepared by inoculating a loopful of test organisms in 5 ml of Nutrient broth and incubated at 37 °C for 18 h. fungal inoculum was prepared by inoculating a loopful of test organisms in 5 ml of Sabouraud's dextrose broth and incubated at 30 °C for 2-3 days.

Preparation of various extracts of algae

An aliquot of 10 g of each alga was extracted successively with 250 ml of hexane, ether, dichloromethane and acetone by using a soxhlet extractor until the extract was clear, apparatus for 6 h. While the same dry weight of algae was extracted by maceration, on dark at 30 °C, with 100 ml methanol/water (7:3) till exhaustion for 24 h and was extracted three times (3 × 100 ml) to harvest the maximum of compounds (Fig. 1).

The extracts were collected, filtered and the filtrate was concentrated under reduced pressure by using a rotatory evaporator at the respective boiling points of the solvents. The extracts were transferred to a hot air oven, where it was dried at 40 °C and stored at 4 °C. Portion of the extract was used for phytochemical analysis while the rest was used for the bacterial susceptibility test.

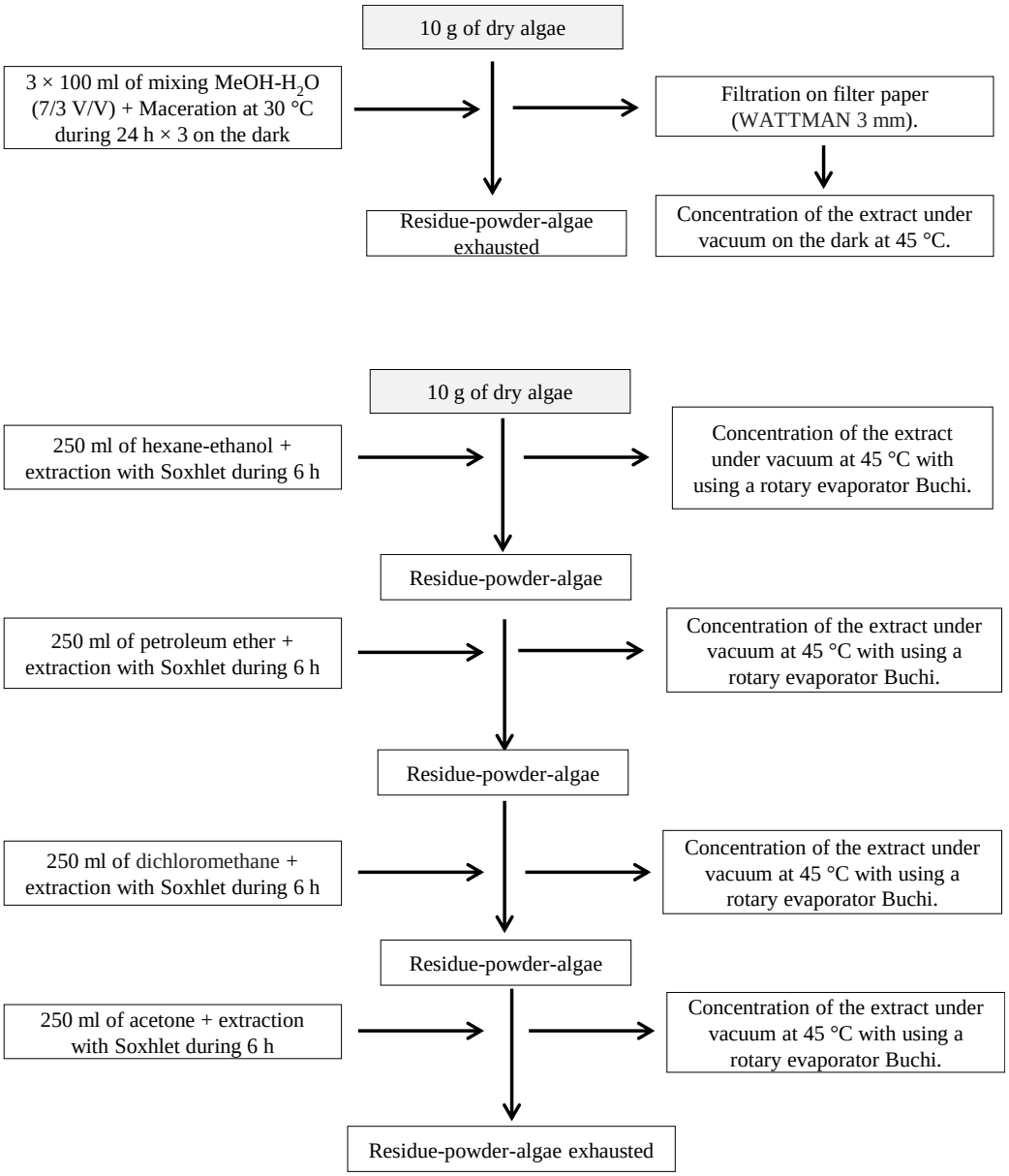

Figure 1. Protocol for the organic extraction of the dry algae.

Antimicrobial Screening by the Disk Diffusion Method

Antibacterial and antifungal activities were checked by the agar diffusion method of Spooner & Sykes (1972) encountering the diameter of the inhibitory zone in the soft agar layer. *In vitro* antimicrobial activity was screened by using Muller Hinton agar plates inoculated with 1 % of test bacterial samples and Sabouraud's dextrose agar plate for fungi.

The disc loaded with extracts (20 mg/100 µl) was placed on the surface of medium and all Petri dishes were kept in the refrigerator (4 °C) for 2 h for the compound was allowed to diffuse. The plates were incubated at 37 °C for a period of 18-24 h for bacteria and 25 °C for 24-48 h for fungi. The discs treated with 100 µL of organic solvents were used as negative controls for each extract and gentamicin, erythromycin were used (10 µg) as positive controls. The extracts containing antibacterial and antifungal components produce distinct, clear, circular zones of inhibition around the discs and the diameters of clear zones were determined in millimeters. Each assay in these experiments was repeated three times for concordance. Toxicity of the solvent extract has been tested by Paper disc diffusion method.

Minimum Inhibitory Concentrations (MIC)

The minimum inhibitory concentration (MIC) was determined against tested microorganisms using agar dilution method recommended by the NCCLS (1999) and Saini *et al*. (2005). MIC was defined as the lowest algal extracts concentration showing no visible bacterial or fungal growth after incubation for 24 h at 37 ºC and 48 h at 25 °C, respectively.

Phytochemical analysis

Phytochemical analysis of the extract was carried out using chemical method and tested for the presence of various phytoconstituents which are followed as protocol as per the methods adopted by Harborne *et al*. (1998).

The total carbohydrate content was determined as previously described in our previous communication (Abdo*et al*. 2012). The chlorophyll was spectrophotometrically determined according to Lichtenthaler (1987) method. The concentration of total blue pigment phycocyanin was spectrophotometrically determined at 280, 615 and 652 nm; respectively as reported by Silverira *et al*. (2007). Phycocyanin concentration (PC) and extraction purity (EP) were calculated by the following equation: (PC) = OD615 – 0.474 (OD 652) / 5.34 mg ml^{-1} and (EP) = OD615 / OD280, respectively.

The total phenolic content of the prepared extract was estimated with Folin-Ciocalteu method (Singleton *et al*. 1965) and the results were expressed as mg of gallic acid equivalent (GAE)/100 mg dry weight. Absorbances were recorded at 750 nm in a UV-VIS spectrophotometer. Total flavonoid content was determined according to Kosalec *et al*. (2004). They were measured spectrophotometrically against $AlCl_3$ solution as quercetin mg/g at 450 nm. The total condensed tannins content were determined with the vanillin in acidic medium method (Prices *et al*.1978) using tannic acid as standard. The results were expressed as tannic acid equivalent (TAE) mg/100 mg dry weight.

Statistical analysis

Data are presented as mean ± standard deviation (SD) of four determinations. Statistical analyses were performed using the one way ANOVA with 95 % confidence limits ($p < 0.05$).

RESULTS AND DISCUSSION

Identification of micro algae

The algal samples were identified based on the morphological identification, the microalgae cells were observed in the optical microscope (Fig. 2) and with scanning electron microscopy (SEM) (Fig. 3).

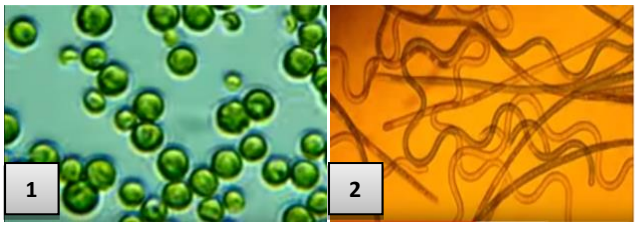

Figure 2. Cells of *Chlorella pyrenoidosa* (1) and *Spirulina platensis* (2) observed in the optical microscope (Gr: 100×1.25×10×0.25)

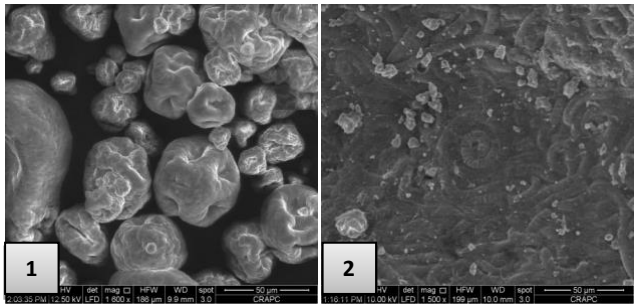

Figure 3. Cells of dried *Chlorella pyrenoidosa* (1) and *Spirulina platens*is (2) observed with the SEM Scanning Electron Microscopy. Magnification is 1600 ×, scale bar = 50 µm.

Colors and yields of the various fractions extracted from *Spirulinaplatensis and Chlorella pyrenoidosa*

The result showed that the methanolic extract of *Chlorella* saves performance highest in the order of 9.97 % followed by the Hexano-ethalonic extract of 8.98 % and 7.53 % for the etheric extract. While we note the largest amount of extraction with methanol solvent (9.88 %) followed by the Hexano-ethalonic extract (8.70 %). The results are displayed in table 1. With regard to the other extracts, the values range from 2.33 and 5.49 % (Tab. 1).

Phytochemical analysis

The results of phytochemical analysis of acetone, methanolic, etheric, dichloromethalonic and hexanic extracts of *Spirulina platensis and Chlorella pyrenoidosa* revealed the presence of flavanoids, saponins, tannins, carbohydrates, phenolics, terpenes and cardiac glycosides. Steroids and alkaloids were absent in all the extracts (Tab. 2).

Tannin, Sterols, terpenoids & quinonic substances were absent in all the extract. Phenolic compounds and flavonoids were present in all the extract (Tab. 2). Alkaloids are present only in acetonic & methanolic extracts. The total phenolics and flavonoids compounds, phycocyanin and chlorophyll concentrations of the two algal extracts are presented in table 3. The highest value of total phenolic was determined in *Chlorella* (106.52 ± 0.25mg/g) followed by *Spirulina* (33.57± 1.11 mg/g).

In addition, the highest value of total flavonoid was noted in *Chlorella* (37.12± 0.94 mg/g) then *Spirulina* (15.35± 0.54 mg/g). The higher concentration of phycocyanin was in *S. platensis* sample and the higher concentration of Chlorophyll in *Chlorella.* These results are in agreement with those reported by Ali *et al*. (2014) in which they observed that *Chlorella* sp. and *Scenedesmus obliquus* presented higher phenolic and carotenoid contents. Microalgae contain a variety of phenolic classes but they were very different from many other plant species like vegetables, fruits and medicinal plants. The microalgae could contain different antioxidant compounds compared to other plants (Manivannan *et al.* 2012). The presence of flavonoids and phenols in the methanol extract might be responsible for free radical scavenging activity individually or by synergistic action. Klejdus *et al*. (2010) showed that several classes of flavonoids, such as isoflavones, flavanones, flavonols and dihydrochalcones are found in microalgae and cyanobacteria. This indicates that microalgae are more primitive than terrestrial plants and they are capable of producing relatively complex polyphenols.

Alkaloids are commonly found to have antimicrobial properties (Omulokoli *et al*. 1997) against both Gram-positive and Gram-negative bacteria (Cowan 1999).

Antimicrobial activities

The result obtained from the present study concerning the antimicrobial activity produced by the cyanobacterium *Spirulina platensis* and the green microalga *Chlorella pyrenoidosa* against four Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC

10876, *Bacillus subtilus* ATCC 6633, *and Bacillus subtilus* ATCC 9372 and four Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella* sp ATCC 4352, *Salmonella typhimurium* ATCC 14028) as well as for their antifungal activity against *Candida albicans* ATCC 10231 and *Aspergillus* sp ATCC 16404 were presented in table 4.

All extracts tested exhibited variable antibacterial activities against microorganisms tested. The *Chlorella pyrenoidosa*-methanolic total extracthas shown a wide spectrum of activity as the highest inhibition zone (48 ± 7.07 mm) at a concentration of 0.96 mg/disk followed by the Hexano-ethalonique extract with 47 ± 5.66 mm (0.96 mg/disk)against *Escherichia coli* ATCC 25922. The MIC values varying from 0.19 to 2.97 mg ml^{-1} and 1.97 to 2.67 mg ml^{-1} respectively (Tab. 5). While the methanolic total extract of *Spirulina* (1 mg/disk) showed 43± 4.24 and 27.5± 0.71 mm, respectively against Gram-positive *B. subtulis* ATCC 6633 and *B. cereus* while it showed 31± 1.41and 27.5± 3.54 mm, respectively against Gram-negative *Pseudomona* and *Klebsiella*. However, the MIC values varying from 0.13 to 1.9 mg ml^{-1} (Tab. 6). Our results were more important than those found by Usharani *et al*. (2015) in which they reported that the methanol crude extract of *Spirulina platensis* showed highest mean zone of inhibition (20± 0.4 mm) against the Gram-positive cocci *Streptococcus pyogenes* followed by *Staphylococcus aureus* (19± 0.3 mm). For Gram-negative bacteria, the maximum zone of inhibition was recorded in methanol crude extract against *Proteus mirabilis* (19± 0.8 mm) followed by *Klebsiella pneumoniae* (19± 0.5 mm). The same results were also obtained by Ozdemir *et al*. (2004), Asthana *et al*. (2006), Kaushik *et al*. (2009), Parisi *et al*.(2009), Sudha *et al*. (2011) and Vinay *et al*. (2011). The minimum zone of inhibition obtained from the etheric extract of *Chlorella* against the majority of pathogenic strains was comparatively very less when compared to the other solvent extracts with MIC values varying from 0.75 to 2.25 mg ml^{-1} (Tab. 5). The zone of inhibition obtained from the dichloromethanolic and acetonic crude extracts of *Spirulina platensis* against *Candida albicans* was similar to the results obtained by Usharani *et al*. (2015). While for *Spirulina*-etheric extract, we registered an antibacterial activity only towards the *Escherichia coli*-strains ATCC 25922, *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeroginosa* ATCC 27853. The Hexano-ethalonic extract presented a high activity towards Gram-negative *Bacillus cereus* ATCC 10876 followed by *Escherichia coli* ATCC 25922. While retrieves it acetone and dichloromethanolic presented anactivity-spectrum as inhibition-zone of the order 23 mm against *Escherichia coli* and *Staphylococcus aureus* respectively.

In this study, it was observed that the all extracts obtained from Cyanobacteria and *Chlorella*-etheric-extract used in this study had a negative antifungal activity toward *Aspergillus brosiliensis* ATCC 16404 with the exception of the etheric and methanolic extracts of the green algae (Tab. 4) who have presented an activity of 13.5 and 20 mm respectively. Whereas, *C. pyrenoidosa* extracts showed 13 ± 1.41, 15.5 ± 0.71 and 21.25 ± 6.99 mm respectively against *Candida albicans* but not record antifungal activity of *Cyanobacteria* extracts. On the other hand, dichloromethanolic and acetonic extracts showed antifungal activity with inhibition zones (13 ± 1.41 and 15 ± 1.41 mm, respectively).These results are in agreement with those obtained by Entesar & Ahmed (2016) who have found that all algal species did not record antifungal activity against *Aspergillus fumigatus* and *Trichophyton mentagrophytes*, while toward *Candida albicans* recorde inhibition zones with 15.1 ± 1.2 mm. The same results were also obtained by Rania *et al*. (2008) who have found that *Chlorella pyrenoidosa*-green microalgae-ethanolic extracts present an antifungal effects to ward *A. flavus* (30 mm) following by acetonic extract (15 mm). While ethanolic extract gave the largest inhibition zones on the plates of the tested fungi with 40 mm toward *A. flavus* following by *C. albicans* (20 mm).

The results indicated that the extract of *Chlorella pyrenoidosa* was the most prominent effect against the tested Gram-positive bacteria and fungi strains while *Spirulina platensis* extracts were more efficient against the tested Gram-negative bacteria. A higher antimicrobial activity in the methanolic extract of *Chlorella* may be due to abundance of some lipophilic, but polar compounds and recorded to the highest percentages of thetotal phenolic and total flavonoid contents. The effect of antimicrobial activity of *Chlorella* species has been reported in other studies such as Kellam & Walker (1989) and Ordog *et al*. (2004) reported that antibacterial and antifungal activities were seen predominantly from the *Chlorella* species. Also Ozdemir *et al*. (2001) found that extracts of *Spirulina* obtained by different solvents exhibited antimicrobial activity on both Gram-positive and Gram-negative organisms. The antimicrobial activity of the extract could be due to the presence of different chemicals that may include flavonoids and triterpenoids besides phenolic that may affect growth and metabolism of bacteria. Also, they could have an activating or inhibitory effect on microbial growth according to their constitution and concentration, compounds and free hydroxyl group (Yu *et al*. 2009) amides and alkaloids (Ghasemi *et al*. 2004). 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 (Lee *et al*. 2007, Mishra *et al*. 2007). It has been suggested that the lipids and fatty acids present in the algal strains could also be responsible for the antimicrobial activity (Demule *et al*. 1996, Lampe *et al*. 1998). Fatty acids isolated from microalgae have been known to exhibit antibacterial activity (Kellam *et al*. 1989).

Table 1. Color and yields of the various fractions extracted from *Spirulina* and *Chlorella* in percentage compared to the total weight of each microorganism powder. Values within each column with different letters (a–e) differ significantly ($p < 0.05$).

Species	Extracts	Color	Mass (g)	Yields (%)
Spirulina platensis	Hexano-ethanolic	Caramel	0.087	8.70[b]
	Petroleum ether	Yellow	0.037	3.70[d]
	Dichloromethalonic	Brown	0.0233	2.33[e]
	Acetonic	Dark green	0.0549	5.49[c]
	Methanolic	Green-bluish	0.0988	9.88[a]
Chlorella pyrenoidosa	Hexano-ethanolic	Caramel	0.089	8.98[b]
	Petroleum ether	Yellow	0.075	7.53[c]
	Methanolic	Green	0.099	9.97[a]

Table 2. Preliminary Phytochemicalanalysis of *Spirulina platensis and Chlorella pyrenoidosa*. + Present, ND: Not detected.

Chemical compounds wanted	Organic extracts				
	Ether	Hexane	Dichloromethane	Acetone	Methanol
Phenolic compounds	+	+	+	+	+
Flavonoids	+	+	+	+	+
Tannin	-	-	-	-	-
Sterols & Terpenoids	-	-	-	-	-
Quinonic substances	-	-	-	-	-
Alkaloids	-	-	-	+	+
Cardiac Glycosides	-	-	-	-	+

Table 3. Qualitative analysis of chemical compounds of organic fractions extracts from *Spirulina platensis and Chlorella pyrenoidosa*. The data refer to mean value ± standard deviation. Values within each column with different letters differ significantly ($p < 0.05$).

Algal species	Composition (mg/g)			
	Total phenol content	Total flavonoid content	Phycocyanin	Chlorophyll
Spirulina platensis	33.57 ± 1.11[b]	15.35 ± 0.54[b]	55.7 ± 1.23[a]	8.12 ± 1.24[b]
Chlorella pyrenoidosa	106.52 ± 0.25[a]	37.12 ± 0.94[a]	40 ± 1.13[b]	12.63 ± 0.9[a]

Table 5. Antimicrobial activity as minimum inhibitory concentration (MIC µg/ml) of crude extract of *Chlorella pyrenoidosa* against tested microorganisms. Data are expressed in the form of mean ± SD; NT: not tested.

Microorganisms	Organics extracts (µg/ml)		
	Etheric	Hexano-ethalonic	Methalonic
E. coli	1500 ± 0.70	2670 ± 0.70	> 198 ± 2.12
S. aureus	2250 ± 1.41	< 1780 ± 2.12	>198 ± 2.12
Salmonella typhimurium	750 ± 0.70	< 1780 ± 2.12	>198 ± 2.12
B. cereus ATCC 10872	2250± 1.41	2670 ± 0.70	< 198 ± 0.70
B. subtulis ATCC 9372	2250± 1.41	>1780 ± 2.12	>495 ± 1.41
B. subtulis ATCC 6633	2250± 1.41	2670 ± 0.70	>2970 ± 2.12
Klebsiella sp	1875± 0.70	2670 ± 0.70	1980 ± 0.70
Pseudomonas aeroginosa ATCC 27853	2250± 1.41	>1780 ± 1.41	2970 ± 0.71
Aspergillus sp	2250± 1.41	NT	>495 ± 1.41
Candida albicans	2250± 1.41	2670 ± 0.70	>495 ± 1.41

Table 6. Antimicrobial Activity as minimum inhibitory concentration (MIC µg/ml) of crude extract of *Spirulina platensis* against tested microorganisms. (NT: not tested). Data are expressed in the form of mean ± SD.

Microorganisms	Organics extracts (µg/ml)					
	Etheric	Dichlorometanolic	Hexano-ethanolic	Acetonic	Methanolic	Gentamycin (µg)
E. coli	> 370	< 233	< 1740 ± 1.41	> 450 ± 0.70	1976	< 100
S. aureus	> 740	< 233	< 1740 ± 1.41	> 450 ± 0.70	1976	
Salmonella typhimurium	NT	NT	NT	NT	1976	NT
B. cereus ATCC 10872	> 740	> 466	< 1740 ± 1.41	> 900 ± 0.70	> 988	< 100
B. subtulis ATCC 9372	> 740	> 466	< 1740 ± 1.41	>1647 ±0.70	128 ± 4,24	< 100
B. Subtulis ATCC 6633	> 740	> 466	< 1740 ± 1.41	>526 ± 0.70	128 ± 0,71	< 100
Klebsiella sp	NT	NT	NT	NT	NT	< 100
Pseudomonas aeroginosa	NT	466	1740 ± 0.70	1647 ± 0.70	2964	< 100
Candida albicans	NT	466	NT	900 ± 0.70	NT	< 100

Table 4. Antibacterial and antifungal activities of crude algal extracts against pathogenic bacteria and fungi (inhibition zone expressed as mm diameter), data are expressed in the form of mean ± SD.

		Diameter of effective zone of inhibition* (mm)									
		E. coli	*Sal. typhimurium*	*Kb.*	*Ps.*	*S. aureus*	*B. cereus*	*B. subtulis*	*B. subtulis*	*A. brosiliensis*	*C. albicans*
S. platensis	Etheric	21.5± 0.71^{d}	17 ± 0.71^{d}	0	11 ± 0.1^{d}	10 ± 0.1^{e}	20 ± 1.41^{d}	20.3 ± 2.12^{c}	19 ± 0.71^{b}	0	0
	Dichlorometalonic	14.5 ± 0.71^{f}	0	0	13.5 ± 0.71^{c}	23 ± 4.24^{b}	12.5 ± 3.54^{e}	10.5 ± 0.71^{f}	12 ± 2.83^{d}	0	13 ± 1,41^{c}
	Hexano-ethanolic	28 ± 2.83^{b}	21.9 ± 1.41^{c}	12 ± 1.41^{c}	10.5 ± 3.54^{d}	27 ± 2.83^{a}	40.5 ± 0.71^{a}	25 ± 2.83^{b}	0	0	0
	Acetonic	23.5 ± 2.12^{c}	0	9.5 ± 2.12^{d}	9.5 ± 0.71^{d}	11.5 ± 0.71^{e}	16.5 ± 2.12^{e}	21.5 ± 0.71^{c}	9.50 ± 0.71^{e}	0	15 ± 1,41^{b}
	Methanolic	16 ± 1.41^{e}	22 ± 0.71^{c}	27.5 ± 3.54^{a}	31 ± 1.41^{a}	21 ± 1.41^{c}	27.5 ± 0.71^{b}	43 ± 4.24^{a}	0	0	0
C. Pyrenoidosa	Etheric	21 ± 1.41^{d}	31 ± 1.41^{a}	11.25 ± 2.47^{c}	13.75 ± 1.06^{c}	13 ± 5.66^{d}	14.5 ± 0.71^{e}	15.5 ± 4.95^{e}	15 ± 1.41^{c}	13.5 ± 0.71^{b}	13 ± 1.41^{c}
	Hexano-ethanolic	47 ± 5.66^{a}	27 ± 0.71^{b}	13.5 ± 2.12^{c}	15.5 ± 0.71^{c}	14.5 ± 3.54^{d}	19.5 ±0.71^{d}	18 ± 2.83^{d}	22 ± 2.83^{a}	0	15.5 ± 0.71^{b}
	Methanolic	48 ± 7.07^{a}	28.6 ± 2.12^{b}	20.3 ± 2.12^{b}	19.3 ± 1.41^{b}	26.3 ± 2.12^{a}	23.6 ± 1.41^{c}	11.5 ± 0.71^{e}	20 ± 1.41^{b}	20 ± 1.41^{a}	21.25±6.99^{a}
Standard antibiotics	GEN (µg)	23	29	nt	nt	26	nt	nt	nt	nt	nt
	ERI (µg)	13	20	nt	nt	18	nt	nt	nt	nt	nt

* Effective zone of inhibition include the diameter of the discs of paper filter (6 mm), results are the means of diameter, *S. platensis : Spirulina platensis, C. pyrenoidosa: Chlorella pyrenoidosa, E. coli: Escherichia coli* ATCC 25922, Sal.: *Salmonella typhimurium ATCC , Kb: Klebsiella* sp ATCC 4352, *Ps.: Pseudomonas aeroginosa* ATCC 27853, *S. aureus*: *Staphylococcus aureus* ATCC 6538, *B. cereus*: *Bacillus cereus* ATCC 10876, *B. subtilus*: *Bacillus subtilus* ATCC 6633, *and Bacillus subtilus* ATCC 9372, A. *brosiliensis*: *Aspergillus brosiliensis* ATCC 16404, C. *albicans :Candida albicans* ATCC 10231. GEN: Gentamycin, ERI: Erythromycin, nt: not tested.

CONCLUSION

The organic extracts of *Chlorella pyrenoidosa* algal strainused in the present investigation showed a most prominent effect against the tested Gram-positive bacteriaand fungi strains while *Spirulina platensis* extracts were more efficient against the tested Gram-negative bacteria, but further researches should be made to identify and purify natural productspresenting these antibacterial and antifungal activities.

An improved knowledge of the composition, analysis, and properties of *S. platensis* and *C. pyrenoidosa* with respect to antimicrobial compounds would assist in efforts for the pharmaceutical application. Having knowledge of impact of infectious diseases on global health and the continued emergence of antibiotic resistance bacteria, the study would help the biopharmaceutical industry in the timely and efficient development of a new product of nutritional interest preventive and therapeutic.

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