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Molecular characterization of two microalgal strains in Egypt and investigation of the antimicrobial activity of their extracts

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The emergence of new pathogens and the increasing drug-resistance of recognized ones pose a difficult challenge. One way that this challenge is being addressed is through the discovery of new cost-effective drug resources in the form of bioactive compounds. Algae represent a promising source of bioactive compounds in this regard. In the present research, we used molecular and phylogenetic analysis to isolate and identify two microalgal strains. We found that one strain belonged to the phylum chrysophyta and the other to the cyanobacteria. We also investigated the antimicrobial activity of some of the lipophilic extracts of the two microalgal strains. Several fractions showed high individual antimicrobial bioactivity against multidrug-resistant *Salmonella* sp., *Citrobacter* sp., *Aspergillus niger* and *Aspergillus flavus*. Fraction III from *Poterioochromonas malhamensis* showed the highest level of activity against two multidrug-resistant bacterial pathogens. The inhibition zone diameter was 1.4 cm for *Salmonella* and 1.4 cm for *Citrobacter*. Meanwhile, another lipophilic fraction from the cyanobacterium *Synechocystis salina* showed broad-spectrum bioactivity (inhibition zone diameter of 0.9 cm for *Aspergillus niger*, 1 cm for *Citrobacter* and 0.9 cm for *Salmonella*). One lipophilic fraction from *Aphanizomenon* showed antifungal bioactivity against *Aspergillus niger* and *Aspergillus flavus*, where the inhibition zone diameter was 1.1 cm and 1.0 cm, respectively. The study highlights the antimicrobial bioactivity of extracts from local microalgae and emphasizes the importance of carrying out screening programs for those microorganisms.
Keywords. Antimicrobial, *Aphanizomenon*, *Aspergillus*, *Citrobacter*, multidrug-resistant, *Salmonella*, *Poterioochromonas malhamensis,* Egypt.

Caractérisation moléculaire de deux souches de micro-algues issues d'Égypte et recherche d'une activité antimicrobienne de leurs extraits. L'émergence de nouveaux agents pathogènes et l'augmentation de la résistance aux médicaments de ceux qui sont connus posent un défi difficile. Des composés bioactifs, économiquement rentables, récemment découverts, sont étudiés à cette fin. Des micro-algues en constituent une source prometteuse. Dans le présent travail de recherche, nous avons pu isoler et identifier deux souches de micro-algues en utilisant l'analyse moléculaire et phylogénétique et nous montrons qu'une des deux souches appartient aux chrysophycées et l'autre aux cyanobactéries. Nous rapportons également une activité antimicrobienne de certains de leurs extraits lipophiles. Plusieurs fractions ont montré un niveau élevé de bioactivité antimicrobienne contre plusieurs organismes résistants à plusieurs médicaments, *Salmonella* sp., *Citrobacter* sp., *Aspergillus niger* et *Aspergillus flavus*. La fraction 3 de *Poterioochromonas malhamensis* a montré le niveau d'activité le plus élevé contre deux pathogènes bactériens résistants à plusieurs médicaments. Le diamètre de la zone d'inhibition était de 1,4 cm pour *Salmonella* et 1,4 cm pour *Citrobacter*. Parallèlement, une autre fraction lipophile de la cyanobactérie *Synechocystis salina* a présenté une activité biologique à large spectre (le diamètre de la zone d'inhibition était de 0,9 cm pour *Aspergillus niger*, 1 cm pour *Citrobacter* et 0,9 cm pour *Salmonella*). Une fraction lipophile d'*Aphanizomenon* a montré une bioactivité antifongique contre *Aspergillus niger* et *Aspergillus flavus*, les zones d'inhibition ayant un diamètre de 1,1 cm et 1,0 cm, respectivement. Cette étude met en évidence l'activité antimicrobienne de micro-algues locales et démontre l'intérêt de la mise sur pied de programmes de dépistage de ces micro-organismes.
Mots-clés. Antimicrobien, *Aphanizomenon*, *Aspergillus*, *Citrobacter*, résistance à plusieurs médicaments, *Salmonella*, *Poterioochromonas malhamensis*, Égypte.

1. INTRODUCTION

Algae are oxygenic phototrophs found in all water bodies worldwide. They comprise different groups of organisms, varying widely in their morphology, anatomy and habitats and capable of surviving in the most extreme niches (Whitton et al., 2000). Microalgae have the ability to produce a wide range of bioactive compounds (Thajuddin et al., 2005), enabling them to cope with their environments and to out-compete other co-existing microorganisms through the antimicrobial effect of those compounds (Mundt et al., 2003). It is well known that the current sources of antimicrobial compounds mostly originate from actinomycetes and plants (Borowitzka, 1995). We are currently witnessing the declining effectiveness of widely used antibiotics and the emergence of new pathogenic microorganisms and/or the increasing resistance of existing ones (Skulberg, 2000). This means that it is becoming increasingly important to search for new sources of bioactive antimicrobial compounds. Cyanobacteria (blue-green algae) are considered to be a rich source of secondary metabolites that has yet to be fully explored (Ehrenreich et al., 2005). According to Skulberg (2000), the pharmaceutical effects and modes of action of cyanobacterial secondary metabolites are diverse (Thajuddin et al., 2005). These compounds include immunosuppressants and toxins, as well as antimicrobial and anti-tumor compounds (Ehrenreich et al., 2005; Barrios-Llerena et al., 2007). The importance of cyanobacteria as a potential drug resource is evidenced by the launch of the "Cyanomyces" project in Europe, which aims to generate novel therapeutic substances by combining genes from actinomycetes and cyanobacteria (http://www.cyanomyces.com) (Asthana et al., 2006). In addition, other microalgae have long been known to be potent sources of antimicrobial compounds (see, for example, Pratt et al., 1944 on *Chlorella*; Rossell et al., 1987 on brown algae). Among the chrysophytes, a chlorophyll-related bioactive compound called malhamensilipin A has been isolated from *Poterioochromonas* (Pereira et al., 2010). Porphyrin forms the core of chlorophyll and derivatives of this lipophilic compound include (Z)-24-propylidenecholesterol, which is proposed as a chemotaxonomic marker for some chrysophytes belonging to the class Pelagophyceae (Giner et al., 1998). Porphyrin derivatives have also been found in dinoflagellates and are thought to play a defensive role whereby their structural modifications make the sterols inedible to marine invertebrates, hindering predation and thereby enhancing the bloom-forming ability of dinoflagellates (Giner et al., 2003).

In Egypt, local strains of microalgae are proving to represent quite promising potent sources of bioactive antimicrobial compounds (Issa, 1999; El-Sheekh et al., 2006), especially lipophilic microalgal extracts (El Semary et al., 2009; El Semary et al., 2010; El Semary, 2012). Our research follows on from these earlier studies by carrying out further screening of microalgae collected from the Helwan Governorate region of Egypt. In the present study, two microalgae were isolated, one belonging to the chrysophytes and the other to the cyanobacteria. First of all, we established the taxonomic identity of these microalgae. We then investigated the antimicrobial bioactivity of lipophilic extracts taken from both these microalgae and from another cyanobacterial strain, against many pathogenic microorganisms, some of them multidrug-resistant strains. The lipophilic extracts from the microalgal strains were then fractionated using column chromatography and the separated fractions were used in antimicrobial bioassay.

2. MATERIALS AND METHODS

2.1. Sampling and establishing microalgal cultures

Water samples were collected in sterile samplers from the River Nile, in the Maadi area, and from a wastewater canal, in Helwan Governorate. Microscopic examination revealed the presence of cyanobacteria, diatoms, chrysophytes and some chlorophytes. Water samples were spun and spread on solid *Oscillatoria* medium (Feuillade, 1994) and ASN medium (50 ml medium: 0.5 gm agar, v: wt). Colonies were identified using light microscopy, and were then purified and used to establish monospecific cultures. Two microalgae were isolated: one a chrysophyte from the River Nile and one a cyanobacterium from Teraat El Khashaab. The two cultures were purified and identified morphologically using light microscopy.

2.2. Taxonomic identification of the two microalgae

Genomic DNA was extracted using a modified method from Smoker et al. (1988). The small subunit rRNA gene was used as a taxonomic marker. For the cyanobacterium, the purified genomic DNA was used as a template for amplification of partial 16S rDNA using cyanobacterial highly-specific primers Cya395 and $781R_{1, 2}$ (Nübel et al., 1997). Almost the full length of 16S rDNA was then obtained using the Cya395 forward primer in combination with the 1492 reverse primer designed by Delong (1992). The taxonomic identity of the two microalgae was verified using Euk forward and Euk reverse primers (Delong, 1992). The 16S rRNA and 18S rRNA gene sequences were deposited in the GenBank database under the accession numbers JF442038 and JF442039.

For the purpose of phylogenetic analyses, cyanobacterial 16S rRNA gene sequences were imported from GenBank and aligned in the Clustal W tool within the alignment function of the MEGA4 phylogenetic package. Similarly, the closely-related 18S rRNA genetic sequences of *Ochromonas* were used to reconstruct phylogenetic trees using different methods integrated into the MEGA4 software (Tamura et al., 2007). Bootstrap values from 500 resamplings were calculated for each set of data and all trees were rooted using the unicellular cyanobacterium *Microcystis aeruginosa* PCC7806 for the cyanobacterium and *Scenedesmus communis* for the *Ochromonas*. All resulting trees had similar topologies, indicating similar phylogenetic relationships. The bootstrapped-consensus maximum parsimony tree was chosen for presentation. The ME tree was searched using the Close-Neighbor-Interchange (CNI) algorithm (Nei et al., 2000). The Neighbor-Joining algorithm (Saitou et al., 1987) was used to generate the initial tree. Phylogenetic analyses were conducted using MEGA4 (Tamura et al., 2007).

2.3. Antimicrobial bioassay

Initial antibiotic susceptibility test. Pathogenic microbes (**Table 1**) were used as test microorganisms in antimicrobial tests (Doan et al., 2000). These test organisms were potent microbial pathogens collected from clinical samples and preserved as pure cultures. Their pathogenic effects are listed in **table 1**. The isolates were identified using traditional biochemical tests (Murray et al., 1995). The tested organisms were subcultured at 37 ºC and were maintained on nutrient agar media (Oxide, UK). Several antibiotics were tested in the initial susceptibility/resistance test. The bacterial isolates were tested for their resistance to antibiotics using a disk diffusion method on Mueller-Hinton agar (Oxide, UK) and were incubated overnight at 37 °C. The antibiotic disks (Oxoid, UK) used were ampicillin (10 µg), cefepime (30 µg), cefotaxime (30 µg), chloramphenicol (30 µg), doxycycline (30 µg), erythromycin (15 µg), levofloxacin (5 µg), rifampicin (30 µg), tobramycin (10 µg), ciprofloxacin (15 µg), amikacin (30 µg) amoxycillin/clavulinic acid (30 µg), oxacillin (30 µg), and ceftriaxone (30 µg). Zones of inhibition were determined in accordance with the National Committee for Clinical Laboratory Standards (NCCLS, 2000); isolates were categorized as either susceptible or resistant (**Table 2**). Results of the antibiotic susceptibility test are recorded in **table 2**.

Antimicrobial bioassay of lipophilic extracts. Microalgal biomass (1 g fresh wt.) was collected from both microalgae as well as from *Aphanizomenon* sp. and the samples were then lyophilized. The lyophilized cells were extracted twice with 100 ml chloroform: methanol (2:1, v: v) HPLC grade for two days, and then centrifuged (14,000 × g) for 30 min using a Hettich-cooling centrifuge (Germany). The supernatant was left to evaporate to dryness and was then re-dissolved in 3 ml chloroform: methanol (2:1, v: v) (modified from Doan et al., 2000). The sample was applied to a Silica gel G60 column (1.5 x 25 cm) prepared from the

Table 1. List of microbial pathogens tested — *Liste des pathogènes microbiens testés*.

Microbial strain and author	Type	Pathogenicity (diseases)
Escherichia coli Migula 1895	Gram negative	Gastrointestinal and urinary tract infection
Citrobacter sp. Werkman & Gillen 1932	Gram negative	Bacteremia associated with malignancies
Salmonella typhi Schroeter 1886	Gram negative	Salmonellosis (food poisoning)
Proteus mirabilis Hauser 1885	Gram negative	Wound infections, septicemia and pneumonias
Staphylococcus epidermidis Winslow and Winslow 1908	Gram positive	Infection in catheters as it grows as biofilms on plastic devices inserted within the body
MRSA *Staphylococcus aureus* Rosenbach 1884	Gram positive	Food poisoning and infection in hospitals (common hospital bug)
Streptococccus faecalis Andrewes & Horder 1906	Gram positive	Endocarditis, as well as bladder, prostate, and epididymal infections
Klebsiella pneumoniae Schroeter 1886	Gram negative	The *Klebsiella pneumonia* disease (human lungs inflammation and hemorrhage)
Pseudomonas aeruginosa Schroeter 1872	Gram negative	Generalized inflammation and sepsis
Aspergillus niger Tiegh 1809	Filamentous fungus	Lung disease (aspergillosis)
Aspergillus flavus Link 1809	Filamentous fungus	Aspergillosis of lungs and sometimes otomycotic, infections

Table 2. Susceptibility tests of bacterial strains to antibiotics — *Tests de sensibilité des souches bactériennes aux antibiotiques.*

Bacterial strains	Resistance / susceptibility test judged by diameter of inhibition zone in mm*								
	Ampicillin	Cefepime	Cefotaxime	Chloramphenicol	Doxycycline	Erythromycin	Levofloxacin	Rifampicin	Tobramycin
Staphylococcus aureus	R	R	R	R	R	R	R	R	R
Staphylococcus epidermidis	R	R	R	R	R	R	R	R	R
Streptococcus faecalis	R	R	R	S	S	R	R	R	R
Escherichia coli	R	R	R	R	R	R	R	R	R
Pseudomonas aeruginosa	R	R	R	R	R	R	R	R	R
Proteus mirabilis	R	R	R	R	R	R	R	R	R
Klebsiella pneumoniae	R	R	R	R	R	R	R	R	R
Salmonella typhimurium	R	R	R	R	R	R	R	-	R
Citrobacter sp.	R	R	R	R	R	R	R	R	R

*Inhibition zones including disc (6 mm) diameter — *zones d'inhibition incluant un disque de 6 mm de diamètre* ; R: resistant — *résistant*; S: sensitive — *sensible*.

slurry from 30 g of precipitated Silica gel G60 (Merck, UK). The lipophilic fractions were collected using the solvent system shown in **table 3**.

The lipophilic extracts were concentrated before being applied to 6 mm paper disks for testing. The paper was left to dry and for the solvent to evaporate before being used in the antimicrobial test. The sensitivity of these pathogenic strains to the extracted fractions was assessed using the slightly modified Kirby Bauer Disk Diffusion Susceptibility method (Bauer et al., 1966). The diameter of the inhibition zone was measured in triplicate and the average and standard deviation recorded (**Table 4**). Disks containing chloroform/ methanol were left to evaporate and were then used as negative controls.

3. RESULTS

3.1. Morphological identification

Both microalgal strains isolated were cocooid cells and were either solitary or aggregated in clumps without a common sheath around them.

3.2. Small subunit rRNA gene molecular analysis and phylogenetic reconstruction

The sequence retrieved was compared with other sequences deposited in GenBank using nucleotide BLAST search. The sequence showed a 97% similarity with the other sequences, with the best relative being *Synechocystis salina* LEGE 06099 (HQ832895). The other best relatives were *Synechocystis Synechocystis salina* LEGE 06155 (HQ832911), and *Synechocystis* PAK-12 (EF429297), both showing a 90% similarity. The phylogenetic analyses using different cyanobacterial representatives and employing different tree reconstruction methods all resulted in trees of similar topologies in which the isolate clustered with *Synechocystis*. They showed a distinct clade that separated away from the out-group taxa of *Microcystis* sp. and *Lyngbya polychroa* LP16 (FJ602745) (**Figure 1**). This clade was supported by a 91% bootstrap confidence value. The *Synechocystis* clade including the strain under study was further sub-clustered from the main sub-clade containing *Synechocystis* strains with a relatively large bootstrap value (67%). The chrysophyte strain showed a 97% similarity to *Poterioochromonas malhamensis* (AB023070), followed by *Poterioochromonas malhamensis* (FN662745), and *Poterioochromonas* sp. Y8 (HM161748). These strains formed a sub-cluster, supported by 99% bootstrap values, from the rest of the *Ochromonas* strains and the out-group taxon *Scenedesmus communis* (**Figure 2**).

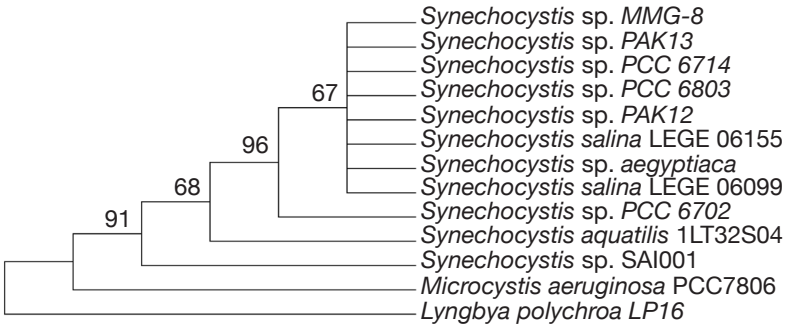

Figure 1. Bootstrap-maximum parsimony tree showing the clustering of *Synechocystis* strain (arbitrarily designated as *Synechocystis* sp. *aegyptiaca*). *Lyngbya polychroa* LP16 FJ602745). *Synechocystis salina* LEGE 06099 (HQ832895). *Synechocystis aquatilis* 1LT 32S04 (FM177503). *Synechocystis salina* LEGE06155 (HQ832911). *Synechocystis* sp. LMECYA 68 (EU078508). *Synechocystis* sp. PAK12 (EF555570). *Synechocystis* sp. PCC 6714 (AB041937). *Synechocystis* sp. PAK13 (EF555571). *Synechocystis* sp. LMECYA 68 (EU078508). *Synechocystis* sp. PCC 6702 (AB041936). *Synechocystis* sp. PCC 6803 (AY224195). *Synechocystis* sp. PCC 6805 (AB041938). *Synechocystis* sp. Sai001 (GU935367) — *Arbre (dendrogramme) du bootstrap selon la méthode de maximum de parcimonie montrant le clustering de la souche* Synechocystis *(aléatoirement désignée par* Synechocystis *sp*. aegyptiaca*)*. Lyngbya polychroa *LP16 (FJ602745)*. Synechocystis salina *LEGE 06099 (HQ832895)*. Synechocystis aquatilis *1LT32S04 (FM177503)*. Synechocystis salina *LEGE 06155 (HQ832911)*. Synechocystis *sp*. *LMECYA 68 (EU078508)*. Synechocystis *sp*. *PAK12 (EF555570)*. Synechocystis *sp*. *PCC 6714 (AB041937)*. Synechocystis *sp*. *PAK13 (EF555571)*. Synechocystis *sp*. *LMECYA 68 (EU078508)*. Synechocystis *sp*. *PCC 6702 (AB041936)*. Synechocystis *sp*. *PCC 6803 (AY224195)*. Synechocystis *sp*. *PCC 6805 (AB041938)*. Synechocystis *sp*. *Sai001 (GU935367)*.

3.3. Antimicrobial bioassay

The preliminary antibiotic sensitivity tests showed the resistance of most of the pathogenic strains to the most commonly used antibiotics (**Table 2**). The only strain showing susceptibility to chloramphenicol and doxycycline was *Streptococcus faecalis*. The lipophilic fractions of *Aphanizomenon* sp. did not show any antibacterial bioactivity against *Citrobacter* sp., *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus epidermidis*, MRSA *Staphylococcus aureus*, *Streptococccus faecalis*, *Klebsiella pneumonia* or *Pseudomonas aeruginosa*. Fraction V showed antifungal bioactivity against both *Aspergillus niger* and *Aspergillus flavus*. Fractions IV and VI showed little antifungal bioactivity against *Aspergillus flavus* (**Table 3**). The lipophilic fractions of *Synechocystis salina* were not effective against *Proteus mirabilis*, *Staphylococcus epidermis*, MRSA *Staphylococcus aureus*, *Streptococccus faecalis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* or the pathogenic fungus *Aspergillus flavus*. The only fraction that was effective against *Aspergillus niger* was fraction V. Two fractions were effective against *Citrobacter* sp. (fractions III and IV). These fractions, together with fraction II, were effective against *Salmonella typhi* (**Table 3**). The lipophilic fractions of

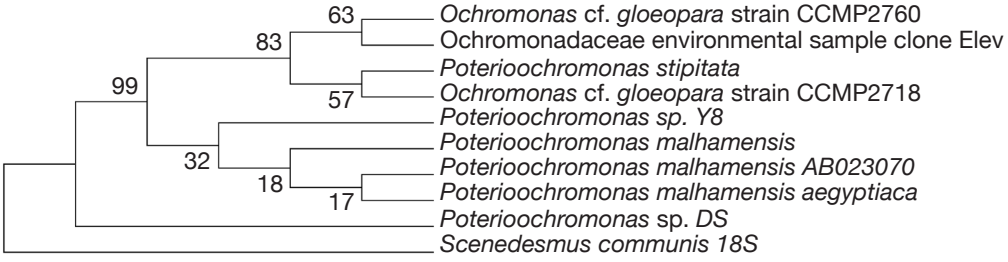

Figure 2. Bootstrap-maximum parsimony tree showing the clustering of *Ochromonas* strains (arbitrarily designated as *Poterioochromonas malhamensis aegyptiaca*). Ochromonadaceae environmental sample clone Elev (EF024948). *Ochromonas* cf. *gloeopara* strain CCMP2060 (EF165113). *Poteriochromonas malhamensis* (AB023070). *Poterioochromonas malhamensis* (FN662745). *Poterioochromonas malhamensis* strain SAG933 (EF165114). *Poterioochromonas* sp. DS (AM981258). *Poterioochromonas* sp. Y8 (HM161748). *Poterioochromonas stipitata* (AF123295). *Scenedesmus communis* (X73994) — *Arbre (dendrogramme) du bootstrap selon la méthode de maximum de parcimonie montrant le clustering des souches Ochromonas (aléatoirement désignée par* Poterioochromonas malhamensis aegyptiaca*)*. *Clone Ochromonadaceae d´echantillon environmental Elev (EF024948)*. Ochromonas *cf*. gloeopara *souche CCMP2060 (EF165113)*. Poteriochromonas malhamensis *(AB023070)*. Poterioochromonas malhamensis *(FN662745)*. Poterioochromonas malhamensis *souche SAG933 (EF165114)*. Poterioochromonas *sp*. *DS (AM981258)*. Poterioochromonas *sp*. *Y8 (HM161748)*. Poterioochromonas stipitata *(AF123295)*. Scenedesmus communis *(X73994)*.

Table 3. Antimicrobial activity of *Aphanizomenon* sp. and *Synechocystis salina* against multidrug-resistant pathogens — *Activité antimicrobienne de* Aphanizomenon *sp. et* Synechocystis salina *contre des pathogènes résistants aux médicaments*.

Solvent system Chloroform: methanol	**Cyanobacteria**	**Fraction** (10 ml)	***Citrobacter* sp.**	***S. typhi***	***E. coli***	***P. mirabilis***	***S. epidermis***	**MRSA *S. aureus***	***S*. faecalis***	***K. pneumoniae***	***P. aeruginosa***	***A. niger***	***A. flavus***
100:00	*Aphanizomenon* sp.	I	-	-	-	-	-	-	-	-	-	-	-
90:10		II	-	-	-	-	-	-	-	-	-	-	-
80:20		III	-	-	-	-	-	-	-	-	-		
70:30		IV	-	0.8 ± (0.1)	-	-	-	-	-	-	-	-	0.7 ± (0.1)
60:40		V	-	-	-	-		-	-	-	-	1.1 ± (0.1)	0.7 ± (0.1)
50:50		VI	-	-	-	-	-	-	-	-	-	-	0.8 ± (0.1)
100:0	*Synechocystis salina*	I	-	-	-	-	-	-	-	-	-	-	-
90:10		II	-	1.2 ± (0.2)	-	-	-	-	-	-	-	-	-
80:20		III	1.0 ± (0.2)	0.9 ± (0.1)	-	-	-	-	-	-	-	-	-
70:30		IV	-	-	-	-	-	-	-	-	-	-	-
60:40		V	-	-	-	-	-	-	-	-	-	0.9 ± (0.1)	-
50:50		VI	1.0 ± (0.1)	0.8 ± (0.1)	-	-	-	-	-	-	-	-	-

S: *Salmonella*; *E*: *Escherichia*; *P*: *Proteus*; *S*: *Staphylococcus*; *S**: *Streptococcus*; *K*: *Klebsiella*; *P*: *Pseudomonas*; *A*: *Aspergillus*.

Poterioochromonas malhamensis were not effective against any fungal strain or against most of the bacterial strains, except for *Citrobacter* sp. and *Salmonella typhi*. Fractions III.1 and III.2 were highly effective against these last two bacterial strains (**Table 4**).

4. DISCUSSION

The identity of the local microalgal strains (*Synechocystis salina*, a prokaryotic coccoid cyanobacterium and *Poterioochromonas malhamensis*, a eukaryotic microalga belonging to the chrysophytes) was confirmed by traditional phenotypic characterization and modern molecular and phenotypic analyses. The high percentage of sequence identities of both local strains and their co-clustering among similar isolates of known identities further confirmed their taxonomic designations. *Poterioochromonas malhamensis* is known for its mixed mode of nutrition whereby phagotrophy is performed in the form of bacteriovory along with phototrophy. This mixed mode of nutrition is very important in aquatic systems as it ensures the high survival rate of this microalga even under stress conditions. It also means that high death rates of bacteria can be attributed to the activity of this microalga (Sanders et al., 1990). Supernatants from microalgal cultures of *Poterioochromonas malhamensis* have been shown to exhibit antibiotic action that may be mostly attributed to lipophilic substances released from this microalga (Blom et al., 2010). In addition, extracts from *Poterioochromonas malhamensis* have shown a broad-spectrum antibacterial effect (Blom et al., 2010). Moreover, the bioactivity of one compound, called malhamensilipin A, isolated from this microalga has been reported (Pereira et al., 2010). Malhamensilipin A acts as an antiviral and antimicrobial agent as well as an inhibitor of the enzyme tyrosine kinase, which also indicates its possible activity as an anticancer agent (Chen et al., 1994). The microalga *Poterioochromonas malhamensis* itself is reported to possess several chlorine-substituted bioactive compounds exhibiting antimicrobial activity (Chen et al., 1994). This may explain the antimicrobial bioactivity of extracts from

Table 4. Column fractionation and the antimicrobial effect of the lipophilic extracts from the microalga *Poterioochromonas malhamensis — Fractionnement en colonne et effet antimicrobien des extraits lipophiliques de la microalgue* Poterioochromonas malhamensis.

Solvent system Chloroform: Methanol (v:v)	Fraction	*Citrobacter* sp.	*S. typhi*	*E. coli*	*P. mirabilis*	*S. epidermis*	MRSA *S. aureus*	*S*. faecalis*	*K. pneumoniae*	*P. aeruginosa*	*A. niger*	*A. flavus*
100:00	I.1	-	1.1 ± (0.1)	-	-	-	-	-	-	-	-	-
	I.2	1.0 ± (0.1)	0.8 ± (0.1)	-	-	-	-	-	-	-	-	-
90:10	II.1	-	-	-	-	-	-	-	-	-	-	-
	II.2	-	-	-	-	-	-	-	-	-	-	-
80:20	III.1	1.4 ± (0.2)	1.4 ± (0.2)	-	-	-	-	-	-	-	-	-
	III.2	1.5 ± (0.2)	1.1 ± (0.2)	-	-	-	-	-	-	-	-	-
70:30	IV.1	-	-	-	-	-	-	-	-	-	-	-
	IV.2	-	1.1 ± (0.2)	-	-	-	-	-	-	-	-	-
60:40	V.1	1.1 ± (0.1)	1.1 ± (0.2)	-	-	-	-	-	-	-	-	-
	V.2	-	-	-	-	-	-	-	-	-	-	-

-: no activity — *pas d'activité*; *S*: *Salmonella*; *E*: *Escherichia*; *P*: *Proteus*; *S*: *Staphylococcus*; *S**: *Streptococcus*; *K*: *Klebsiella*; *P*: *Pseudomonas*; *A*: *Aspergillus*.

this microalga against multidrug-resistant strains in the present study.

The second microalgal strain under study, *Synechocystis salina*, was isolated from a highly-polluted wastewater canal into which industrial, agricultural and domestic wastes are known to be released. This has resulted in water of a brackish nature (conductivity value of 8,091 $\mu S \cdot cm^{-1}$) (Hamed, 2008) and the prevalence of stressful conditions that select for microalgae with unique metabolic features, enabling them to survive and thrive (El Semary, 2012). Interestingly, in his study of Egyptian water habitats, including water bodies in the Helwan area, Hamed (2008) found that *Synechocystis salina* thrived within the brackish-saline-hypersaline range of conductivity in a wide range of habitats. This indicates the possibility that the metabolic adaptability of this cyanobacterium is mediated through the possession of unique metabolites.

Our results also showed the extreme resistance of most of the isolates tested to the most commonly used antibiotics. The emergence of microbial multidrug-resistance to antibiotics is attributed to many complex factors, such as mutations, genetic recombinations and geographical segregation (Hujer et al., 2006). Microbial multidrug resistance represents a widespread problem nowadays, especially where antibiotics have been intensively used (Piddock, 2002). Pathogenic bacteria develop resistance by acquiring genes that encode proteins to protect them from the effects of the antibiotic. In some cases, the genes arise through mutation, but in others they are acquired from other bacteria that are already resistant to antibiotics. These genes are often found on plasmids, which spread easily from one bacterium to another (Fluit et al., 2001; Bhakta et al., 2003).

In the present study, although extracts were relatively effective against some pathogens, no single extract had a broad-spectrum effect against all the pathogenic strains studied. Nevertheless, a few extracts showed some cross antimicrobial bioactivity against different microorganisms, *e.g.* fraction IV of *Aphanizomenon* sp. (**Table 4**). El Semary (2009) conducted phytochemical screening for *Aphanizomenon* sp., showing that the bacterium possessed alkaloids and flavonoids, and that it had a relatively high lipid content as well as a high level of iron. Interestingly, in the same study, molecular screening for the presence of genetic loci of secondary

metabolites was positive for *Aphanizomenon* sp., indicating its potentiality as a producer of bioactive compounds and as a possible antibiotic resource. This hypothesis was then confirmed by the current investigation.

In conclusion, the extracts from the microalgae studied here showed considerable antimicrobial bioactivity against several multidrug-resistant isolates. Screening programs need to be extended further in order to discover more bioactive compounds from local microalgal strains in Egypt.

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