BIOLOGICAL AND PHYTOCHEMICAL STUDIES ON SOME ISOLATED USEFUL ALGAE FROM SIWA OASIS

Submitted By

Youssra Essam Moustafa Abd El Rahman

B.Sc. of Science (Microbiology/Chemistry), Faculty of Science,
Ain Shams University, 1999

A Thesis Submitted in Partial Fulfillment
Of
The Requirement for the Master Degree
In
Environmental Sciences

Department of Environmental Basic Sciences
Institute of Environmental Studies and Research
Ain Shams University

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APPROVAL SHEET

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ABSTRACT

In compliance to the recent surveys on algal species and their potentials to produce biologically active natural products**,** collection of the water samples were carried out during summer 2014 from five water sources in Siwa Oasis. Some physico-chemical and biological parameters for the water samples were recorded. Among these certain physico-chemical parameters were within the acceptable limit in the context of World Health Organization (WHO), while others exceeded. The phytoplankton community was represented by **54** species belonging to **35** genera and **4** algal groups: Bacillariophyta(**20**species), Cyanophyta (**19**species), Chlorophyta (**13** species) and Euglenophyta (**2**species). The results showed a correlation between some physico-chemical parameters and phytoplankton community. Bacillariophyta dominated in Ain Fetnas**,** while Cyanophyta in Ain Cleopatra. Fetnas Lake had the lowest number of total algal taxa mainly due to the exceeded levels of electrical conductivity, Na^{+}, K^{+}**,** Cl^{-}, HCO_3^{-}, Ca^{++}, Mg^{++}, total hardness and Boron. Three common microalgal isolates including one green alga (***Chlorella vulgaris*****)** and two blue green algae **(*Chroococcus turgidus*** and ***Phormidium tenue*****)** were purified using BG-11, Basal Bold and Zarrouk media. Phytochemical analysis revealed the presence of important metabolites (carbohydrates, proteins, sterols, terpenoids, cardiac glycosides, phenolics, flavonoids and saponins) but alkaloids, tannins and anthraquinones were absent in all isolates. Determination of carbohydrates, proteins and vitamin E (α-tocopherol) concentrations were recorded. ***Chlorella vulgaris*** showed the highest concentration value of **31**gm/100gm for carbohydrates and **145.7** ppm for α-tocopherol, while ***Phormidium tenue*** showed the highest value of **28.4**gm/100gm for proteins concentration. The methanolic extracts of the three algal taxa were tested for the antibacterial activity using agar well diffusion method against eight human pathogenic bacteria, two G+ve bacteria ***Staphylococcus aureus*** and ***Enterococcus* sp.** and six G-ve bacteria ***Acinetobacter baumannii, Escherichia coli, Klebsiella***

pneumonia, Proteus* sp.*, Pseudomonas aeruginosa and ***Salmonella tyhphi.*** The results revealed that ***Phormidium tenue*** showed the highest promising antibacterial activity. The in vitro antioxidant potential of the three methanolic extracts were determined using the 1,1-dipheny-2-picryl-hydrazyl radical (DPPH) scavenging assay. ***Phormidium tenue*** showed the highest percentage value of **60.9**% with moderate activity. ***Phormidium tenue*** was choosed for successive extraction using petroleum ether, ethyl acetate, 80%methanol and water solvents to assay their antibacterial activity against the tested bacterial isolates. The ethyl acetate successive extract showed the highest antibacterial activity and was analyzed chromatographically. Results indicated that the phenolic compounds were the common bioactive compounds which were identified and quantified by using the high performance liquid chromatography (HPLC). The results showed the highest concentration for ethyl vanillate compound (**1755.2** $\mu g g^{-1}$) follwed by chatechin (**627.9** $\mu g g^{-1}$).

KEY WORDS: *Siwa oasis, microalgae, water quality, Phytochemistry, Antibacterial activity* and *antioxidant activity*.

Contents

List of Figures

List of Tables

List of Plates

INTRODUCTION

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This research work was achieved on some local aquatic fresh water microalgae isolated from Siwa Oasis. Simply, Siwa Oasis is one of the aforementioned places that didn't undergo before for any comprehensive bioactivity, while **Shaaban (1985)** studied the algal assemblages and species composition of Siwa Oasis and their relatively comparable habitats only. Extensive worldwide search is presently undergoing to find novel therapeutically cheaper alternatives for natural products which can be used in the preparation of drugs (**Mayer *et al.*, 2005** and **Cardozo *et al.*, 2007**). Since 1970s the interest in medicinal plants increased tremendously due to the failure of modern medicine to provide effective treatment for chronic diseases. Since the mid-1980s, a great number of screening programs have been intiated worldwide by pharmaceutical industries, universities and national research institutions with the aim of searching for new bioactive natural medicinal products. Sources of them have expanded to include higher and lower plants and marine organisms, as well as animals that are promising enough to be developed into new drugs (**Wanger, 2005**). Following the glow of the new vision and the greater interest to the pivotal role played by algae and their healing potency.

Literatures specifically which devoted to algal assemblages and species composition of the Egyptian oases and their relatively comparable habitats were very limited e.g: (**Shaaban and El Habibi, 1978; Gharib, 2004; Fawzy *et al.*, 2013** and **Hifney *et al.*, 2013** (in El-Kharga Oasis)**; Shaaban, 1985 (**in Siwa Oasis**); Ibraheem and Al-Sherif, 2009** (in El-Bahariya Oasis)**; Shaaban and Hamed**, **1997** (in El-Arish Valley and its vicinity North Sinai); **Hamed**, **2005** and **2008**

interested in diatoms and cyanobacteria from some hot springs and other different habitats and **Piatek *et al*., 2009** investigated chrysophytestomatocysts (at Ain Sukhna)).

Microphytes or microalgae are microscopic algae, typically found in freshwater and marine systems. Microalgae have been used for therapeutic applications for several years and represent aunique opportunity to discover novel metabolites. The rate of finding metabolites already obtained from other biological sources is less in microalgae as compared with other microorganisms (**Olaizola, 2003)**. Many microalgal extracts were found to have antialgal (**Hellio *et al*., 2002**), antifouling (**Bhadury and Wright, 2004**), anti-allergic (**Na *et al*., 2005**), anti-inflammatory (**Abedin and Taha, 2008**), anticoagulant (**Dayong *et al*., 2008**), anticancer (**Kim *et al*., 2011**), antioxidant activities (**Devi *et al*., 2011**), antibacterial or antifungal (**Jaritz *et al*., 2011**) and antiviral (**Kim *et al*., 2012**). It is worthwhile screening the bioactivity of the microalgal crude extracts for the antibacterial activity against some infectious human pathogenic bacteria. Bacterial infection causes high rate of mortality in human population and aquaculture organisms. For example, ***Salmonella* sp.** causes diarrhea and typhoid fever (**Leven, 1987** and **Jawetz *et al*., 1995**). ***Escherichia coli***, ***Staphylococcus aureus*** and ***Pseudomonas aeruginosa*** cause diseases like mastitis, abortion, respiratory complications including some life threatening illness (**Boyd, 1995**). According to recent reports of the World Health Organization (WHO) it is mandatory to develop safe, nontoxic and efficient anti-bacterial agents of valuable practice in pharmacology and to diminish side effects of antibiotics as the latter could raise toxicities and serious threatened conditions in some cases. On the other hand, 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 (**Walsh, 2003**). The first investigation on antibiotic activity of algae was carried out by **Pratt *et al.*(1944).** Many studies have been established to prove the antimicrobial effect of metabolites extracted from algal species especially those derived from blue green algae (**Zulpa *et al*., 2003; Abedin and Taha, 2008)**. Many investigators have reported antibacterial activities of microalgae as due to fatty acids (**Cooper *et al*., 1983; Findlay and Patil, 1984**). Antibacterial activity of volatile extracts of ***Spirulina platensis*** have been studied by **Ozdemir *et al*. (2004). El-Sheekh *et al*. (2006)** showed that phenolic compound from ***Nostoc muscorum*** exhibited antagonistic activity against Gram +ve and Gram-ve bacteria**.**

A lot of scientific research is focused on exploring safe and effective antioxidant compounds. Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen formed in mitochondria as a natural byproduct of energy production during the oxidative phosphorylation process. Ordinarily, the levels of free radicals in living organisms are controlled by a complex set of antioxidant defenses, which minimize oxidative damage to important biomolecules. Oxidative stress conditions are caused by endogenous oversized formation of ROS that exceeds the availability of antioxidants and also by impact of external stressors. Excessive ROS can induce apoptosis and cause damage, especially to cellular proteins, polyunsaturated fatty acids and DNA. Further, oxidative stress may be associated with nearly 200 diseases, such as cardiovascular diseases, cancer, atherosclerosis, hypertension, ischemia, diabetes mellitus, hyperoxaluria, neurodegenerative diseases (Alzheimer's and Parkinson's), rheumatoid