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Aquatic microalgae *"Anabaena oryzae"*: phenolic compounds, antioxidant activity and antibacterial activity against *Streptococcus mutans* oral bacteria

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ABSTRACT

Natural antioxidant replacements have been a common subject in recent decades for the substitution of artificial antioxidants. Microalgae have been reported to exhibit interesting bioactive properties especially their antioxidant capacity and antibacterial activity. The goal of the present work is to investigate the possibility of using the blue-green microalgae *'Anabeanae orayzae'* for extract preparation and to determine the phenolic compounds that make up this extract and its possible antioxidant and antibacterial capacities by emphasizing the role of the extract in oral disease prevention. The results showed that *Anabaena* extract possesses a notable antioxidant capacity and also good phenolic compounds content. Algae and cyanobacteria have been demonstrated to produce secondary metabolites with diverse bioactivities. The DPPH scavenging activity of different concentrations of *Anabaena* extract. As for the DPPH scavenging activity of different concentrations of *Anabaena* extract, the results show that a concentration of *Anabaena* extract equal to 2.98 mg/ml was capable of inhibiting 50% of the free radicals (IC_{50}). *Anabaena* extract can be thus considered as an excellent antioxidant since its IC_{50} is a small concentration value and it is agreed that a low IC_{50} value represents high antioxidant activity. Concerning the phenolic compounds detected in *Anabaena* extract, they were salicylic acid, gallic acid, tannic acid, caffeic acid, and benzoic acid. The highest concentrations determined in *Anabaena* extract were for salicylic and tannic acids. In regard to *Anabaena* extract antibacterial activity against *S. mutans*, the ability of *Anabaena* extract to inhibit *S. mutans* was tested and the results showed that the extract has an inhibitory effect against *S. mutans* with an inhibition zone diameter equal to 13 (mm). It can be concluded that *Anabaena* extract possesses the potential antibacterial substances that can be used against oral pathogens and for dental caries prevention.

INTRODUCTION

Currently, algae products are extensively explored for their numerous health benefits. Algae are photosynthetic organisms which live in all Earth's aquatic ecosystems. Blue-green algae also known as cyanobacteria are prokaryotic. Secondary cyanobacterial

metabolites have essential properties **(Rashad *et al*., 2018; Yücer *et al*., 2018; Rashad and El-Chaghaby, 2020)**. These aquatic microorganisms represent a vast source of metabolites such as alkaloids, carbohydrates, flavanoids, pigments, phenols, saponins, steroids, tannins, terpenes and vitamins **(Rashad *et al*., 2019)**. Cyanobacteria are believed to be a rich source of polyphenolics **(Singh *et al*., 2017)**, antioxidants and antimicrobials **(Seddek *et al*., 2019)**.

The phenolic compounds found in algae are interrelated with their antioxidant activity **(Seddek *et al*., 2019)**. Phenolic compounds include many classes of substances and can be divided into simple phenols and polyphenols. Phenolic compounds include coumarins, phenolic acids including hydroxybenzoic acids and hydroxycinnamic acids, tannins, esters of gallic acids, flavonoids of which the most important are flavones, isoflavones, flavonols, flavanols, flavanones, chalcones and anthocyanins **(Bogdan *et al*., 2020)**.

Natural sources of antioxidants and antimicrobials are of high use preference especially in food and in medicine. The role of antioxidants and antimicrobials in delaying food spoilage and also their role in avoiding some dental diseases is quite important. Many oral problems are associated with the presence of reactive oxygen species (ROS). Periodontal disease and dental caries are widely spread chronic conditions affecting people worldwide **(Mufeed *et al*., 2014; Sarangarajan *et al*., 2017)**. ROS are implicated in the pathogenesis of periodontal disease as they are released by leukocytes in response to the chronic inflammation which causes oxidative damage to the gingival and the periodontal tissues and the alveolar bone **(Sarangarajan *et al*., 2017)**. Also, the imbalances in levels of free radicals, reactive oxygen species, and antioxidants in saliva play an important role in the onset and development of dental caries **(Mufeed *et al*., 2014)**. At the same time, it is known that oral cavity contains great diversity of microorganisms and despite the fact that these microorganisms are normally considered non-pathogenic but at certain conditions they may lead to diseases, such as dental caries and periodontal diseases **(Araújo *et al*., 2019)**. In such case it is highly important to use an antibacterial agent to avoid such dental problems.

In this context, the aim of the present work is to investigate the possibility of using the blue-green microalgae "*Anabeanae orayzae"* for the preparation of extract and to determine the phenolic compounds composing this extract and its potential antioxidant and antibacterial capacities with emphasizing the role of the extract in the prevention of oral diseases.

MATERIALS AND METHODS

Extract preparation

The microalgae *"Anabaena oryzae"* used in the present study was produced under controlled cultivation conditions in the Agricultural Research Center, in Egypt. For extract preparation: 50g of dried microalgae were extracted in 200 ml of absolute ethanol

(HPLC grade). The extraction was done for 1 hr using an ultrasonic bath at room temperature. The obtained extract was concentrated using a rotary evaporator set at 40°C **(El-Chaghaby *et al*., 2019)**.

Analysis of Phenolic compounds

The phenolic compounds contained in *Anabaena* extract were determined by HPLC Agilent 1260 infinity equipped with C18 column and diode array detector. Standards of quercetin, pyrogallol, cinnamic acid, salysilic acid, gallic acid, benzoic acid, caffeic acid and tannic acid were obtained from Sigma-Aldrich. The determination of phenolic compounds was done following the procedure given by **(Athmouni *et al*., 2015)**

Determination of antioxidant capacity, total phenols and total flavonoids

The total antioxidant capacity of the extract was determined by the "phosphomolybdenum method" using ascorbic acid as standard **(Prieto *et al*., 1999)**. The total phenols content of the extract was determined using the Folin-Ciocaleau method using gallic acid as standard **(Turkmen *et al*., 2006)**. The total flavonoids content of the extracts was determined by the "aluminium chloride" test **(Mohdaly *et al*., 2010)** using quercetin as standard.

Determination of free radical scavenging activity

The free radical scavenging activity of the extract on the stable radical 1, 1-diphenyl-2- picrylhydrazyl (DPPH) was also evaluated as earlier reported by **Aliyu *et al*., (2013)**. Different extract concentrations were tested and the IC_{50} defined as the amount of antioxidant material required to scavenge 50% of free radical in the assay was calculated as mg/ml.

Determination of antibacterial activity

The antibacterial activity of *Anabeana* extract was tested against oral bacterial strain: *Streptococcus mutans* by using the disc diffusion method **(Zhou *et al*., 2018)**.

All analysis of sample were determined with three replicates and the mean data ± standard deviation were computed using the Microsoft excel program for windows.

RESULTS AND DISCUSSION

Antioxidants change the progress of oral problems by compromising antioxidant capacity of crevicular fluid and plasma **(Aksakalli, 2013)**. In the present work, the antioxidant capacity and total phenolic compounds content of *Anabaena* extract are given in table (1). The results showed that *Anabaena* extract possess notable antioxidant capacity and also good phenolic compounds content. Algae and cyanobacteria have been demonstrated to produce secondary metabolites with diverse bioactivities **(Seddek *et al*., 2019)**. The antioxidant capacity of the extract could be mainly attributed to its phenolic compounds that are secondary metabolites described as radical scavengers because they are donors of hydrogen atoms or electrons, producing stable radical intermediates **(Jerez-Martel *et al*., 2017)**. According to **Abd El-Aty *et al*., (2014)** algae usually have high

antioxidant activity owing to their high contents of non-enzymatic antioxidant components, including ascorbic acid, reduced glutathione, phenols, and flavonoids.

Table (1): Antioxidant properties of *Anabaena* extract

	TAA ppm AAE	**TP ppm GAE**
Mean ±S.D.	563.8 ±19.07	349.4 ±42.59

TAA: total antioxidant and **TP:** total phenolic

The antioxidant activity is a complex process, usually happening via several mechanisms. Thus evaluation of plant extracts antioxidant activity is better to be tested using more than one test **(Lefahal *et al.*, 2018)**. In this respect, the free radical DPPH scavenging assay was also employed to assess the antioxidant activity of *Anabaena* extract and the results are depicted in Figure1.

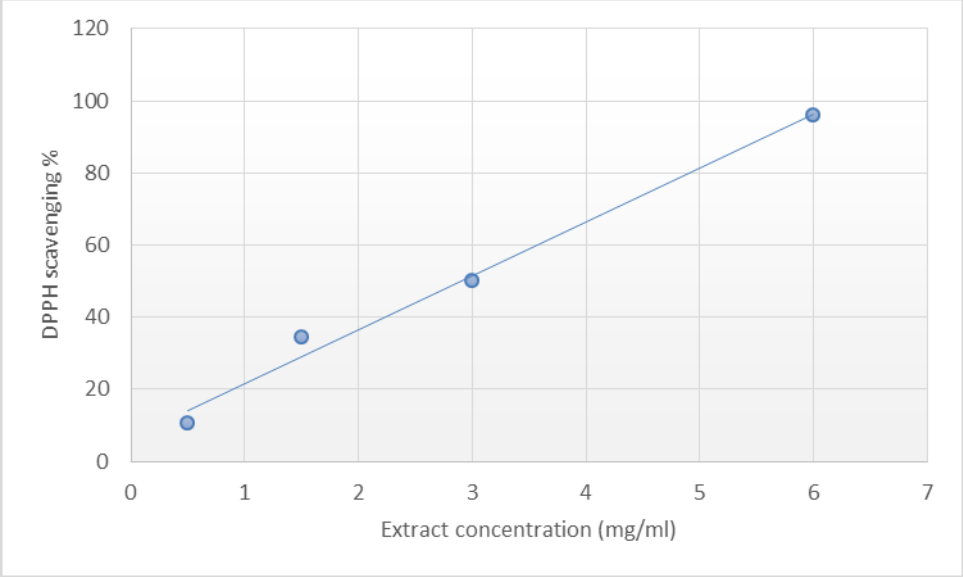

Figure1: DPPH scavenging activity of *Anabaena* extract

Figure 1 shows the DPPH scavenging activity of different concentrations of *Anabaena* extract. It is obvious that by increasing the extract concentration its ability to scavenge DPPH free radicals increased. Results show that a concentration of *Anabaena* extract equal to 2.98 mg/ml was capable of inhibiting 50% of the free radicals (IC_{50}). *Anabaena* extract can be thus considered as excellent antioxidant since its IC_{50} is a small concentration value and it is agreed that a low IC_{50} value represents high antioxidant activity **(Lefahal *et al.*, 2018)**.

Polyphenols, because of their significant features such as antibacterial and antioxidant activities, have an important function to play in the oral cavity against certain illnesses, pathogens and oral cancers **(Kharouf *et al.*, 2020)**. In the present study, the

phenolic compounds present in *Anabaena* extract were determined by HPLC analysis and the confirmed compounds are given in Table (2). The phenolic compounds detected in *Anabaena* extract were salicylic acid, gallic acid, tannic acid, caffeic acid and benzoic acid. The highest concentrations determined in *Anabaena* extract were for salicylic and tannic acids. Salicylic acid is a phenolic plant hormone that was reported to inhibit the proliferation of oral flora **(Kolpan and Bartelink, 2019)**. Tannic acid is a polyphenol that have been reported for its several applications in dentistry as a desensitizing agent, astringent, and surface treatment for smear layer removal **(Bedran-Russo *et al*., 2009)**.

Caffeic acid has proven to have anti-inflammatory activity which is very important in the case of dental caries that causes acute reversible pulpitis **(Alba *et al*., 2016)**. Gallic acid and its esters are hydroxybenzoic derivatives, which are used as antioxidant and its importance in dentistry has been previously confirmed **(Christopher *et al*., 2016)**. Benzoic acid has emerged as an effective method of denture cleansing and has inhibitory effects against the colonization of bacteria on the surfaces of dentures**(Arafa, 2016)**.

Table (2): Quantitative content of phenolic compounds in Anabeana extract

Phenolic compound	**Concentration (ppm)**
Salicylic acid	586518.5
Gallic acid	282.49
Tannic acid	12956.52
Caffeic acid	2201.97
Benzoic acid	850.05
Butaylated hydroxyl toluene (BHT)	Not detected
Quercetin	Not detected
Pyrogallol	Not detected

Anabaena* extract antibacterial activity against *S. mutans

The gram-positive bacteria *Streptococcus mutans* are present in the human mouth, more precisely, in multi-species biofilms on the surface of the teeth. These bacteria are the main cariogenic organisms resulting from their ability to generate vast amounts of glucans as well as acid, increasing the salivary buffering capability, which gives the bacteria an advantage over noncariogenic commensal species in low pH conditions. **(Metwalli *et al*., 2013)**. Decreasing the bacterial load of the oral cavity is thus one of the

basic biological strategies for the prevention of dental caries **(Wassel and Khattab, 2017).**

In the present work the ability of *Anabaena* extract to inhibit *S. mutans* was tested and the results are illustrated in Figure 2. It was observed that the extract has an inhibitory effect against *S. mutans* and showed an inhibition zone diameter 13 (mm). The antibacterial activity of the extract could be largely attributed to the different phenolic compounds determined by HPLC analysis. It is generally agreed phenolic compounds involve many sites of action at the cellular level and many researchers explained this phenomenon by modifying the permeability of cell membranes, by modifying the various intracellular functions caused by hydrogen binding of phenolic compounds to enzymes or by altering the rigidity of the cell wall with integrity losses due to various interactions with the cell membrane. **(Bouarab-Chibane *et al.*, 2019)**.

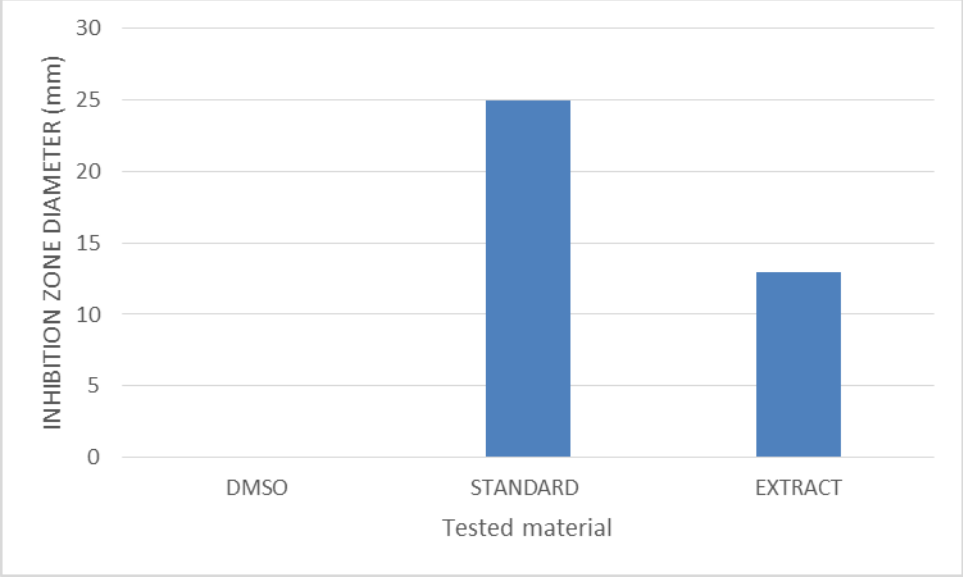

Fig. 2: Antibacterial activity of *Anabaena* extract against *S. mutans*

CONCLUSION

In conclusion, Microalgae have been reported to exhibit interesting bioactive properties. *Anabaena* extract was proved to possess notable antioxidant capacity and also good phenolic compounds content along with its inhibitory effect against *S. mutans*. It can conclude that these *Anabeana* extract possess the potential antibacterial substances that can be used against oral pathogens.

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