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Activity Ethanolic and Methanolic Extracts of Arthrospira Platensis on Pathogenicity of Staphylococcus Aureu Sand Streptococcus Mutans

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Abstract: This study is found outthe bioactive compounds in *Arthrospira platensis* alga that is obtained fromthe Environmental Research Center\ University of Technology. The extraction process is done with ethanol 99% and methanol 99% as solvents. *Staphylococcus aureus* and *Streptococcus mutans* are obtained from Al-Ameen Center for researchand advanced biotechnology in the Holy City of Najaf from urine. which is gram-negative To evaluate the antibacterial activity are used four concentrations (0.5, 1.5, 3, 15) mg\ml for each extract with three replicates by agar well diffusionmethod.

As a result, all concentrations make bacterial inhibition zone and affected more by increasing the extract concentration and highest inhibition rate is for ethanolic extract with an (15.08 ±2.38mm) while the methanolic extract with an average (14.28±2.53mm) And appeared cultivated on Congo Red Agar medium is able tochange the medium color from pink or red to black, indicating their ability to form biofilms, and the ethanolic extract had an anti-biofilm effect.

Key words: Activity ethanolic**,** methanolic extracts**,** pathogenicity

INTRODUCTION

Arthrospira platensis considers as a food source due to high levels of protein and fatty acids, besides carbohydrates, vitamins, and minerals (Watson and Preedy, 2019). In addition to its role against pathogenic bacteria, it can secrete chemicals that have a positive effect on the immune system (Han *et al.*,2021). Excessive use of antibiotics caused by some bacteria and fungi has led to the emergence of strains that are resistant to many bacterial infections and are of little benefit in addition to their high cost and side effects such as hypersensitivity and depletion of the necessary neighborhoods in the gut (Jena and Subudhi, 2019) For these properties and their importance, the effectiveness of compounds in microalgae against bacteria is studied by preparing crude extracts of it. Many ***Staphylococcus aureu sand Streptococcus mutans*** cells are encapsulated or microencapsulated, with acidic polysaccharides serving as the capsules. ***Staphylococcus aureu sand Streptococcus mutans*** mucoid strains contain extracellular slime made up of either a polysaccharide with particular antigen specificities or a common acid polysaccharide made up of colonic acid (often referred to as M antigen) (Jimenez *et al*., 2012). It can be found as normal florain the intestinal tract of animals, and humans transfer to the environment

through excrement and wastewater treatment (Berthe *et al*., 2013). ***Staphylococcus aureu sand Streptococcus mutans*** biofilms on surfaces of mari**ne habitats, such as sediments,**are renowned factors in survival in natural environments (Lee *et al*., 2006). Also are found to be the major causative agent of many intestinal infections. Biofilms provide protection from UV radiation, dehydration, bugs, and antibiotics, among other things. They can also be used to provide microorganisms with nutrients. In aquatic environments, higher flowrates can remove bacteria from existing biofilms, leading in the formation of new biofilms. (McDougald *et al*., 2012**).**

2-Materials and methods:

2-1reparation of *Arthrospira platensis* samples

Large amount of *A. platensis* is obtained from the University of Technology, Environmental Center, Baghdad.

the samples were put in 25 ml flasks with 10 ml distilled water and 4-5 drops of Lugol's iodine Solution added

preservative,(Prescott *et al*., 1996).

2-2 Preparation of *A. platensis* extracts

1- Mixing 5 g of the powder of *A. platensis* with 250 ml of 99% ethanol, the mixture was stirred carefully for 24

2- hours separatly on a magnetic stirrer, thena reflux process was performed for 16 hours and filtered with

3- filter paper (Wattman No. 1), that, concentrating using a rotary evaporator at a degree of 50 C° , obtained a solid substance weighted 1.6 g (Al-Aarajy, 2012)

2-3-Antibiotics

Table (1) Antibiotics used in current study with their manufacturerand origin

No.	Antibiotic name	Icon	Concentration	Manufacturer name
1	Cefixime	CFM	10 mcg	Bioanalyse
2	Cephalexin	CL	30 mcg	Bioanalyse
3	Doxycycline	DO	10 mcg	Bioanalyse
4	Meropenem	MEM	10 mcg	Mast group LTD
5	Oxacillin	OX	10 mcg	Bioanalyse
6	Trimethoprim	TMP	5 mcg	Biolab

2-4 Preparation of Culture Media

Cultures can be prepared by following these steps:

1-Weigh the ready-to-dry medium powder, according to the instructions on it middle container.

2-Dissolve it in the volume of distilled water specified in the instructions, andit will be dissolved

using the flask, and then stirred, and heat the water to dissolve completely.

3-Sterilize the medium by autoclave for 15 minutes under a pressure of 15 lbs and temperature at

121°C.

4- Take it out of the device and wait for it to cool down a bit and pour it into sterilized Petri dishes near

Bunsen burner to prevent contamination and the dishes remain open until the steam does not condense

on the plate lid after freezing it is kept in the refrigerator, then keep in the refrigerator until use (Mac

Faddin, 2000

Table (2) The cultural media used with their manufacturing company and function.

No.	Culture media	Manufacturing company and Origin	function
1	Brain heart infusion agar	Biolab- Hingary	This media was used fordetection the capability of bacteria to biofilm formation (Tille, 2017) .
2	Muller- Hinton agar	Himedia- India	Antibiotic sensivity test(Atlas, 2010).

2-5 -Detection of biofilm formation

The detection of biofilm production by isolated bacteria in current study was detected by the Congo Red

Agar method that is specially prepared by adding (10 g\ l) sucrose to (37\l) g of brain heart infusion agar

mixed with an aqueous solution of Congo red stain (0.8 g/l) that was autoclaved at 121ºC for 15minutes.

the plates were inoculated with selective bacteria that was tested theturbidity with McFarland solution then

incubated at 37°C for 24 hours. The appearance of black dry crystalline colonies on the CRA plates indicated

biofilm production while the colonies of biofilm nonproducer remained pink orred-colored (Sultan and Nabiel, 2019)

3- Results and discussion

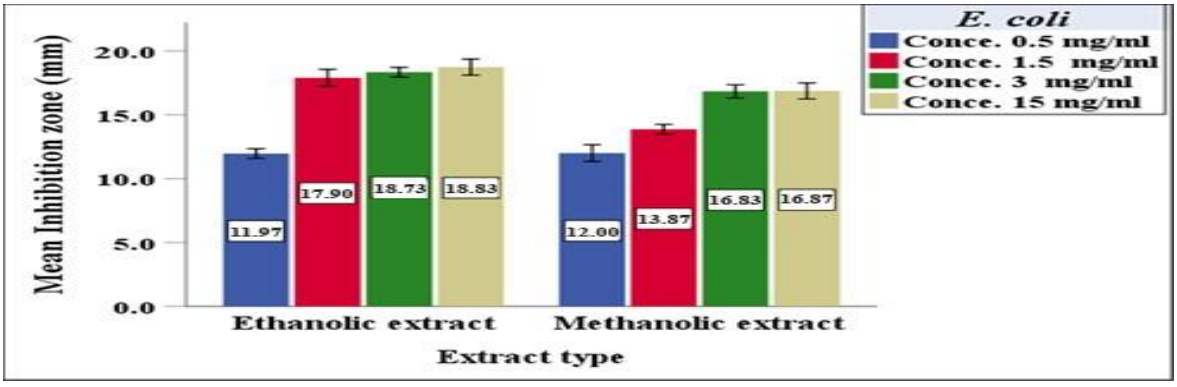

Figure (1) Effect of ethanolic and methanolic extracts of *A. platensis* on Inhibition zone of *Staphylococcus aureu sand Streptococcus mutans*

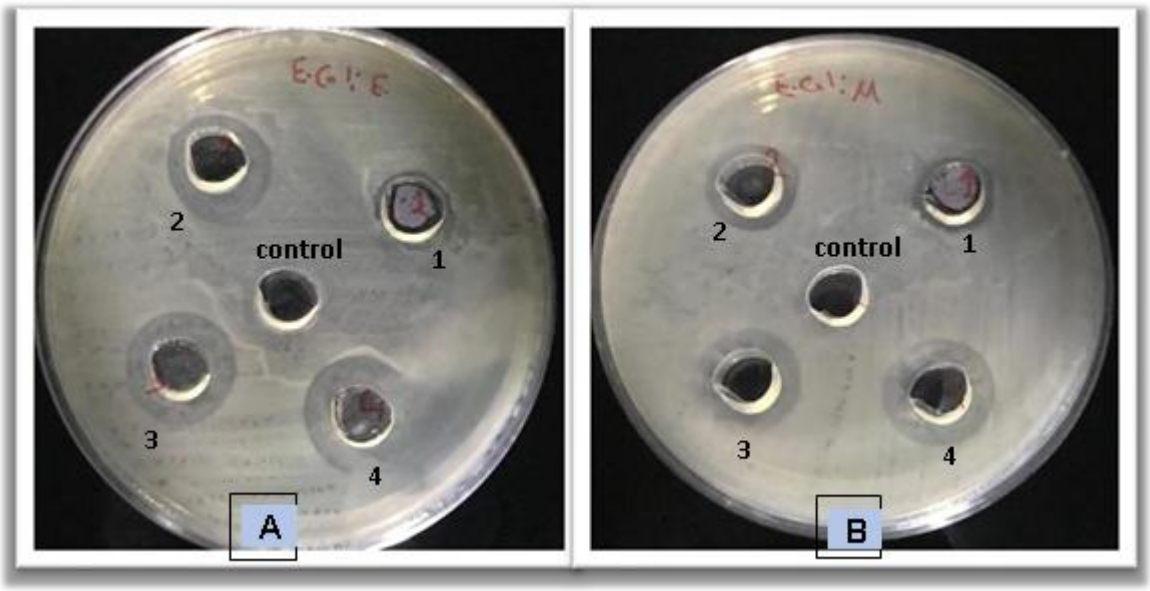

Figure (2) Inhibition zone of *Staphylococcus aureu sand Streptococcus mutans* with *A. platensis*:

A: ethanolic extract **B: methanolic extract**

1:0.5mg\ml **3:3mg\ml**

2:1.5mg\ml **4:15mg\ml**

The antibacterial activity has been examined at different concentrations of ethanolic and methanolic extracts as well as a control group by using the disc diffusion method with eight pathogens. According to different studies the antibacterial activity in *A. platensis* refers to the presence of different compounds (Ozdemir *et al*, 2004). Figure (1) and (2) showed the effect of ethanolic and methanolic extracts of *A. platensis* on the inhibition zone of *Staphylococcus aureu sand Streptococcus mutans*, where the highest valueof inhibition is 18.83 mm on ethanolic extract in concentration 15 mg/ml, whilethe lowest inhibition value is 11.97 mm in concentration 0.5 mg/ml, whereas the higher inhibition zone in the methanolic extract is 16.87 mm in concentration 15 mg/ml and the lower inhibition zone is 12.00 mm in concentration 0.5 mg/ml. When comparing between two extracts, the ethanolic extract had the highest antibacterial activity at a concentration of 15 mg/ml withan average (18.83 ±0.15) at a P-value (0.0001) (Appendix3-A). This result is compatible with Shaieb *et al*., (2014) which mentioned that ethanolic extract of*A.platensis* has antibacterial activity to *E.coli*. While Manigandan and Kolanjinathan, (2017) disagree with this result and cleared that methanol extract of *A. platensis* showed maximum zone of inhibition against *Staphylococcus aureu sand Streptococcus mutans* due to the presence of phytochemical compounds such as protein, carbohydrates, flavonoids, phenols, and terpenoids which exhibits the relation to the antimicrobial activity of *A. platensis* against human pathogens. ppearance of the inhibition zone surrounding the well, this indicates its anti- biofilm effectiveness.

Table(3) Effect of ethanolic and methanolic extracts of *A.platensis* on inhibition zone on *Staphylococcus aureu sand Streptococcus mutans.*

Inhibition zone (mm) of *Staphylococcus aureu sand Streptococcus mutans* (A)			
Extract Concentration	Ethanolicextract	Methanolicextract	Univariate p-value
Conce. 0.5 mg/ml	11.97 ±0.15 C	12.00 ±0.26 C	0.0001 ***
Conce. 1.5 mg/ml	17.90 ±0.26 B	13.87 ±0.15 B	
Conce. 3 mg/ml	18.73 ±0.25 A	16.83 ±0.21 A	
Conce. 15 mg/ml	18.83 ±0.15 A	16.87 ±0.25 A	
LSD	0.399	0.421	
p- value	0.0001 ***	0.0001 ***	

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