Evaluation of antibacterial activity of three extracts of *Spirulina platensis on Streptococcus salivarius*

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Abstract

In this study, antibacterial properties of three extracts of *Spirulina* powder extract on *Streptococcus salivarius* were studied. Two methods including Agar Disk Diffusion and Microdilution were used for evaluation of antibacterial activities. The inhibition zone results were methanol> ethanol> acetone extracts. The MIC and MBC results for methanol extract were 175 and 200 ppm respectively. However, MIC for ethanol extract was 200 ppm while acetone was negative effect on *S. salivarius*.

Spirulina platensis, Streptococcus salivarius, antibacterial, MIC & MBC

Materials and Methods

Algal sample of *Spirulina platensis* for the present investigational study was obtained from the Caspian Research Group of Fisheries and Water Pollutants, Sari, Iran.

Procedure for the preparation of *Spirulina* powder extract

Various extracts prepared from 25 g algae powder which is placed at 250 ml with 3 different solvents (Ethanol alcohol 95%, acetone and methanol) , mixed with each other that is placed at Soxhlet extractor apparatus for 24 hours ,the effective algae materials were separated. The obtained extract being strained and was filtered through a 20 µm Whatman paper filter as well, is concentrated using a R-300 Rotavapor at 45 ° C temperature and dried at OVEN during 48 hours.

Preparation of bacterial suspension, evaluation of antibacterial activity in Agar Disk Diffusion and Microdilution methods

For comparison, evaluation of the agar well was carried out for antibacterial algae (*S. platensis*) strength effects which were placed by extracts,10 micro liter of bacterial suspension at conventional tubes (equal to 1.5×10^8 CFU/ml) are super facially cultured on Brain Heart Infusion broth at medium .(final concentration at bacterial inoculation is equal to 1.5×10^6 CFU/ml and then after wells with 6 millimeter diameter yield, the sterile pipette pastor was provided on surface medium . In the end experiment, 50 ul of stocks was provided on ethanol, methanol and acetone from *S. platensis*, their concentration (150, 170, 175 and 200 ppm) for the provided wells was added and the plates were inoculated at 35 c temperature for 48 hours at microaerophilic conditions. Determination MIC and MBC from *S. platensis* using microdilution method, it is utilized for determination of minimal inhibitory and minimal bactericidal concentrations of *S. platensis* extracts.

Results and Discussion

The results of this study indicate the appropriate antibacterial effect of *Spirulina* powder extract of *Streptococcus salivarius*. The antibacterial activity of *S.platensis* against oral bacteria is shown in Table 1. The methanol extract of *S.platensis* showed maximum inhibition on *Streptococcus*

salivarius at 200 ppm concentration and minimum at 170 ppm concentration. The Ethanol extract of *S.platensis* showed inhibition on *S. salivarius* only at 200 ppm concentration while acetone was but negative effect towards *S. salivarius*. Many investigators mentioned that the acetone extract of cyanobacteria revealed antibacterial activity on *E. coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*(Ishida et al., 1997; De Mule et al.,1991; Vijayakumar et al .,2011). The results also proved that acetone and ethanol were the best solvents for extracting the antibacterial and antifungal agents from *Lyngbya martensiana* and *Oscillatoria latevirens*. In this study methanol was the best solvent for extracting the antibacterial (*S. salivarius*) from *S. platensis*. In this study the amounts MIC (175ug/ml) for S. salivarius and MBC (200ug/ml) in methanol extract. While MBC only showed in (200ug/ml) value. The lack of growth halo was indicated bacterial in acetone extract.

Table1. The inhibition zones results of three solvents extracts of *Spirulina platensis* on *Streptococcus salivarius* in Agar Disk Diffusion method

Streptococcus salivarius	**Type of solvent and its value**	
R	150	Methanol
10.4 ± 0.6	170	
16.0± 0.2	175	
21.0± 0.6	200	
R	150	Ethanol
R	170	
R	175	
18.5± 0. 6	200	
R	150	Acetone
R	170	
R	175	
R	200	
37± 0.7	60	Mouth wash

R: Resistant

In order to determination of MIC (Minimal Inhibitory Concentration) and MBC (Minimal Bactericidal Concentration), 50 u/l from above extracts with one milliliter from bacteria which were surveyed or registered to added test tube ,the test tube was agitated by shaker and then the test tube was surveyed for opacity and transparency after 24 hours as the performed experimental results shown(Table2) .

Table 2. The MIC and MBC results on methanol, ethanol, acetone extracts of *Spirulina platensis* on *Streptococcus salivarius* in Microdilution method

Streptococcus salivarius	**Type of solvent and its value**	
G	150	Methanol
G	170	
MIC	175	
MBC	200	
G	150	Ethanol
G	170	
G	175	
MIC	200	
G	150	Acetone
G	170	
G	175	
G	200	
MBC	60	Mouth wash

G : growth

Conclusion

It can be concluded from the results that methanol extract of *S.platensis* belonging to Cyanobacteria group, used in the present investigation possess significant antibacterial activity against the tested oral bacteria. However, the active components responsible for the antimicrobial activities against oral bacteria need to be evaluated further. *S.platensis* has potential for the development of antibacterial agents against oral pathogens, for use in food additives, mouth-washes, chewing gums and for preventing and treating dental caries.

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