The effect of *Spirulina platensis* extracted by Ultrasound assisted method on Oral streptococci bacteria

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Abstract

Ultrasound-assisted solvent (UAS) has received considerable attention for the recovery of different compounds from various sources. The aim of this study was to evaluate antibacterial activity of the UAS of *Spirulina platensis* on oral streptococci bacteria. Dried powder of spirulina was added to solvents (1: 10 w/v) and extraction was done for 30 min at 45C. The mixture was sonicated at 30 kHz for 15 min at 40C. The concentrations used include 150, 170, 175 and 200 ppm. Two streptococci bacteria include *Streptococcus salivarius* and *Streptococcus mutans* were examined for antibacterial activity. The results showed methanol extract had maximum affect both Agar Disk Diffusion and Microdilution methods. *Streptococcus mutans* was more resistance to *Streptococcus mutans* against used solvents. However, both bacteria were susceptible to methanol extract.

Key words: *Streptococcus salivarius* , *Streptococcus mutans*, *Spirulina platensis*, Ultrasound-assisted solvent

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.

Ultrasound-Assisted Extraction

Dried powder of spirulina was added to three solvents treatments (ethanol, methanol and acetone) (1:10 w/v). The suspensions were shaken in a water bath for 30 min at 45C. The mixture was sonicated with an ultrasonic probe system (Branson, 8510R-Mt, Ontario, Canada) at 40 kHz for 15 min at 40C. The extracts were filtered through Whatman No. 1. The organic solvent was concentrated by using rotary evaporator (Buchi EL 141, Flawil, Switzerland) under reduced pressure (Albu *et al*. 2004).

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 µl of stocks was provided on Ultrasound-assisted 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. For isolation of oral streptococci bacteria was used from

Mitis Salivarius agar. After identification of dominant bacteria, antibacterial activity of UAS of Spirulina was surveyed on *Streptococcus mutans* and *Streptococcus Salivarius.*

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 Ultrasound-assisted methanol and ethanol of *S.platensis* showed better results to acetone extract. In 200 ppm was observed maximum inhibition on both bacteria. *Streptococcus mutans* was more resistance than *Streptococcus salivarius* to used UAS. 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*.

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

Streptococcus salivarius	***Streptococcus mutans***	**Type of solvent and its value**	
R	R	150	Methanol
12.5 ± 0.5	R	170	
18.0± 0.5	15± 0.7	175	
25.0± 0.3	18.5± 0.3	200	
R	R	150	Ethanol
R	R	170	
13.5± 0. 5	R	175	
20.5± 0. 3	17.5± 0.4	200	
R	R	150	Acetone
R	R	170	
R	R	175	
R	R	200	
37± 0.7	32± 0.6	60	Mouth wash

R: Resistant

The results of MIC (Minimal Inhibitory Concentration) and MBC (Minimal Bactericidal Concentration) in microdilution method was similar to Agar Disk Diffusion and UA methanol was more inhibitory effect than on used bacteria in compare to ethanol and acetone extracts (Table2).

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

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

G : growth

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