Comparison of agar disk diffusion and microdiluation methods of solvents extracts *Spirulina platensis* on *Streptococcus mutans*

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Abstract

The present study was comparison of agar disk diffusion and microdiluation methods of solvents extacts *Spirulina platensis* on *Streptococcus mutans*. The natural habitat of *S. mutans* is the human mouth, more specifically dental plaque, where the bacterium resides in multispecies biofilms that form on the surfaces of teeth. While a normal inhabitant of the oral cavity, *S.mutans* is mostly known for its importance in the aetiology of dental caries and occasional association with subacute infective endocarditis. Concerning the antibacterial effects in agar disk diffusion method, the results clearly indicated that methanol and ethanol (value 200) extracts of *S. platensis* had the highest antimicrobial activity with 17.5± 0.5 and 14.5± 0.6mm inhibition zone, respectively. However, in microdiluation method, 175 and 200 ppm were MIC and MBC for methanol extract and 200 ppm was MIC for ethanol extract of *S. platensis* The results also proved that methanol was the best solvent for the extracting the antibacterial (*S. mutans*), while acetone was but negative effect towards *S. mutans*.

Keywords: *Spirulina platensis*, *Streptococcus mutans*,, 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

It is clear from the study that the diameter of the inhibition zone depends mainly on the type of solvent (Table 1).Concerning the antibacterial effects, the results clearly indicated that methanol and ethanol (value 200) extracts of *S. platensis* the highest antimicrobial activity against 17.5± 0.5 and 14.5± 0.6 mm, respectively. These results go in harmony with those obtained (Volk & Furker, 2006) who found that some microalgae had high biological activity against *Bacillus subtilis, B. thungiensis* , *Bacillus megaterium, E. coli, Pseudomonas aeruginosa Candida tropicalis* and *Sauatromyces cerevisiae*. The results also proved that methanol was the best solvent for the extracting the antibacterial (*S. mutans*), while acetone was but negative effect towards *S. mutans*. 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. mutans*) from *S. platensis*. The minimal inhibitory concentration (MIC) and the *Minimum Bactericidal Concentration (MBC)* were determined by serial dilution assay (Table2).

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

Streptococcus mutans	**Type of solvent and its value**	
R	150	Methanol
R	170	
13± 0.5	175	
17.5± 0.5	200	
R	150	Ethanol
R	170	
R	175	
14.5± 0.6	200	
R	150	Acetone
R	170	
R	175	
R	200	
32± 0.6	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 mutans* in Microdilution method

Streptococcus mutans	**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

References

Volk, R.B. and F.H. Furkert, 2006. Antialgal, antibacterial and antifungal activity of two metabolites produced and excreted by cyanobacteria during growth. Microbial. Res., 161: 180-186

Ishida, K., H. Matsuda, M. Murakami and K. Yamaguchi, 1997. Kawaguchipetin B an antibacterial cyclic undecapeptide from the cyanobacterium Microcystis aeruginosa. J. Nat. Prod., 60: 724-726.

De Mule, M., G. De Caire, M. De Cano and D. Haperin, 1991. Bioactive compound from *Nostoc muscorum* (Cyanobacterium). Cytobios, 66: 169-172.

Vijayakumar Madhumathi, Pitchai Deepa, Savarimuthu Jeyachandran,Chockaiya Manoharan and Subramaniyan Vijayakumar. 2011. Antimicrobial Activity of Cyanobacteria Isolated from Freshwater Lake. International Journal of Microbiological Research 2 (3): 213-216, 2011.

Darah I, Lim SH, Ninthianantham K (2013). Effects of methanolic extract of *Wedelia chinensis* Osbeck (Asteraceae) leaves against pathogenic bacteria with emphasize on *Bacillus cereus*. *Indian Journal of Pharmaceutical Science*, 75:533-539.

Goud MJP, Seshikala D, Charya MAS (2007). Antibacterial activity and biomolecular composition of certain fresh water microalgae from River Godavari (India). *Science World Journal*, 2(3): 19-23.

Malathi T, Ramesh Babu M, Lalitha Kumari K, Digamber Rao B (2015). Antimicrobial Activity of Soil Cyanobacteria *Cylindrospermum majus*. *International Journal of Recent Scientific Research*, 6(5):3859-3863.