

## Optimization of Sucrose Concentration, pH Level and Inoculum Size for Callus Proliferation and Anti-Bacterial Potential of *Stevia rebaudiana* Bertoni

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**Abstract :** Background: *Stevia rebaudiana* B. is a shrubby perennial herb of Asteraceae family that possesses the unique ability of accumulative non-caloric sweet steviol glycosides (SGs). Purpose: The purpose of the study is to optimize sugar concentration, pH level, and inoculum size for inducing the callus with optimum growth and efficient antibacterial potential. Method: Three different experiments were conducted in which Callus explant from three-months-old already established callus of *Stevia rebaudiana* of four different sizes was inoculated on Murashige and Skoog (MS) basal medium supplemented with five different sucrose concentration and pH adjusted at four different levels. Results: Maximum callus induction 100, 87.5, and 85.33% resulted in the medium supplemented with 30 g/l sucrose, pH maintained at 5.5, and inoculated with 1.25g inoculum, respectively. Similarly, the highest fresh weights 65.00, 75.50, and 50.53 g/l were noted in a medium fortified with 40 g/l sucrose, inoculated 1.25g inoculum, and 6.0 pH level, respectively. However, the callus developed in a medium containing 50 g/l sucrose was found to be highly antibacterial potent with 27.3 and 26.5 mm inhibition zone against *P. vulgaris* and *B. subtilis*, respectively. Similarly, the callus grown on a medium inoculated with 1.00 g inoculum resulted in maximum antibacterial potential against *S. aureus* and *P. vulgaris* with 25 and 23.72 mm inhibition zone, respectively. However, in the case of pH levels, the medium maintained at 6.5 pH showed maximum antibacterial activity against *P. vulgaris*, *B. subtilis*, and *E. coli* with 27.9, 25, and 23.72 mm, respectively. The ethyl acetate extract of *Stevia* callus and leaves did not show antibacterial potential against *Xanthomonas campestris* and *Clavibacter michiganensis*. In the entire experiment, the standard antibacterial agent Streptomycin showed the highest inhibition zones among the rest of the callus extract; however, the pure dimethyl sulfoxide (DMSO) caused no inhibitory zone against any bacteria. Conclusion: From these findings, it is concluded that among various levels, sucrose @ 40 g L<sup>-1</sup>, pH 6.0, and inoculums at 0.75 g were found best for most of the growth and quality attributes, including fresh weight, dry weight, and antibacterial activities and therefore can be recommended for callus proliferation and antibacterial potential of *Stevia rebaudiana*.

**Keywords :** *Stevia rebaudiana*, Steviol Glycosides, callus, *Xanthomonas campestris*

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