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Production Optimization and Characterization of Anticancer Enzyme L-Asparaginase From *Bacillus Sp.* Using *Moringa Oleifera* Seeds

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EXTENDED ABSTRACT

L-Asparaginase (L-asparagine amino hydrolase 3.5.1.1) constitutes one of the most biotechnologically and bio medically important group of therapeutic enzymes accounting for about 40% of the total worldwide enzyme sales [1, 2]. During current research L-asparaginase, was produced from Bacillus sp. Cultural parameters affecting the production of L-asparaginase were optimized [3]. Maximal yields of L-asparaginase were recorded from 5-day-old culture grown in nutrient broth in presence of Moringa Oleifera seeds as substrate with initial pH 7.0 at temperature 37 ◦ C [4, 5]. Ammonium chloride (1%) and galactose (1%) served as good nitrogen and carbon sources for L-asparaginase production, respectively. Na+and K+ slightly enhanced the productivity of L-asparaginase [6]. The in vitro antioxidant and antitumor activity of partially purified L-asparaginase enzyme was also studied [7]. The enzyme showed a good scavenging activity against DPPH with IC50 value of 81.645µg/mL [8].

To determine anticancer activity, different concentration of partially purified L-asparaginase was tested on heLa cell line by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay [9, 10]. The enzyme showed a significant anti-proliferative activity and a dose dependent effect was observed. The cytotoxicity assay was employed to determine the cytotoxic effects of partially purified enzyme from A9 bacteria on HeLa cell lines. The cell lines were exposed to varying concentration of 100, 50, 25, 12.5, 6.25 and 3.125 µg/mL of test samples for 24 hours. Percentage of cell viability was calculated. It can be observed from figure 1 that all samples exhibited dose-dependent inhibition towards the cell lines used. The percentage of cell viability decreases as the samples concentration increase. L-asparaginase reduced the viability of the cancer cells. At low concentration of 3.125 µg/mL the viability of tumour cells reduced to 89.987% (cell death 10.013%). The dead cells reached to 66.255 % at 100 µg/mL. The IC_{50} value was found to be 69.826 µg/mL.

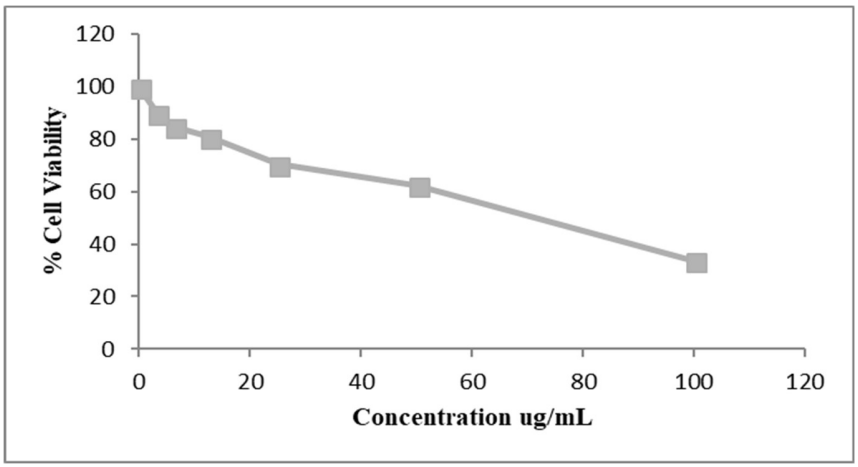

Fig. 1: Cytotoxic effect of A9 L-asparaginase on HeLa cancer cell line at 24 h incubation

Moringa olefiera seeds were used as natural substrate to produce anticancer enzyme L-asparaginase enzyme by fermentation using *Bacillus sp.* Seven parameters carbon, nitrogen, pH, temperature, substrate concentration, inoculum volume and incubation period were optimized for enhanced production of L-asparaginase. Effect of various effectors and antioxidant properties of partially purified enzyme were studied. Furthermore, L-asparaginase also exhibited a cytotoxic effect against cancer cell line *in vitro.* In conclusion, high catalytic activity of the enzyme over a wide range of pH and temperature and its considerable stability makes it highly favorable for use as potent anticancer agent.

Keywords: L-asparaginase; *Bacillus sp*; antitumor activity; *Moringa olefiera.*

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References
[1] Amena, S., et al. (2010) Production, purification and characterization of L-asparaginase from Streptomyces gulbargensis. Brazilian journal of Microbiology, 41(1): p. 173-178.
[2] Singh, Y. and S. Srivastava. (2012) L-asparaginase production by a new isolate *Bacillus aryabhattai* strain ITBHU02 in solid state culture. 1st International Conference on Biosciences and Bioengineering: A collaborative Approach.
[3] Yadav, S., et al. (2014) Industrial production and clinical application of L-asparaginase: A chemotherapeutic agent. Stroke, 76: p. 41.
[4] Narayana, K., K. Kumar, and M. Vijayalakshmi. (2008) L-asparaginase production by *Streptomyces albidoflavus*. Indian Journal of Microbiology, 48(3): p. 331-336.
[5] Singh, Y. and S.K. Srivastava. (2013) Statistical and evolutionary optimization for enhanced production of an anti leukemic enzyme, L-asparaginase, in a protease-deficient *Bacillus aryabhattai* ITBHU02 isolated from the soil contaminated with hospital waste. Indian Journal of Experimental Biology, 51(4): p. 322-335.
[6] Ghosh, S., et al. (2013) Optimization of L-asparaginase production by *Serratia marcescens* (NCIM 2919) under solid state fermentation using coconut oil cake. Sustainable Chemical Processes, 1(1): p. 9.