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ORIGINAL ARTICLE **OPEN ACCESS**

Efficacy of Nano-Zno Conjugated L asparaginase and its anticancer activity against HepG2

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ABSTRACT

This research work aimed to isolate and evaluate Pharmaceutical valuable enzyme L-sparaginase from marine actinomycetes. The phylogenetically identified strain submitted to genebank and accession number is MZ254763 belong to Micromonospora sp *was selected and showed positive results on L asparaginase production by agar plate method. Extracellular protein was isolated and separated by native PAGE and the size of asparaginase was 70 kDa acted as capping agent for reduction of Zinc oxide nanoparticles. Synthesized nanoparticles confirmed at 465 nm and found to be less than 60 nm spherical shapes. The mono dispersed AgNp EDAX confirms it free from other metal ions. Further the enzyme showed maximum activity between pH 7 to 8. The in vitro anticancer against HepG2 reveals that the enzyme zno conjugated enzyme have potential to inhibit proliferation of cancerous cell effectively than asparginase.*
Keyword: Enzyme, Nanomedicine, cancer cell, asparaginase

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INTRODUCTION

Natural reservoir like marine source plays critical role in drug development in current research specially on cancer drugs [1]. Nanotechnology, which involves the production and use of nanomaterials, is growing rapidly and has a wide range of applications in various fields [2]. Different methods have been developed for synthesizing metal nanoparticles including mechanical, chemical, plant and microbial synthesis. The latest has significant advantages over other methods, such as environmental compatibility, synthesis at ambient temperature and pressure, and precise control of size and shape [3]. Metal nanoparticles are used as effective nanocarriers for targeted delivery of anticancer drugs to cancer cells.Metal nanoparticles such as zinc-oxide nanoparticles, iron-oxide nanoparticles, copper-oxide nanoparticles, silver nanoparticles and gold nanoparticles are used as nanocarriers for therapeutic applications due to increases the drug efficacy by sustainable release [4]. The use of metal nanoparticles for the treatment of breast, lung, colon and prostate cancers is advanced the targeting cancerous cells in cancer treatment. Treatment with metal nanoparticles suppresses the expression level of protein B-cell lymphoma in cancer cells hampering its differentiation and proliferation. l-asparaginase will provide synergetic effect in killing cancer cells due to the inherent anticancer properties of l-asparaginase combined with selenium nanoparticles. Asparaginase catalyses the asparagine present on the surface of cancer cells to l-aspartate and ammonia thereby reducing their concentration on the cell surface[5] .Silver nanoparticles are one of the most attractive metal nanoparticles which have antimicrobial, adjuvanticity and anti-tumour properties [6-7]. Moreover, silverbased nanoparticles have attracted attention, because they show enhanced physicochemical and biological characteristics based on the type of additional elements used in the nanoparticle preparations. The half-life of circulating L-asparaginase is drastically reduced (from 18 to 24 h to approximately 2.5 h) due to the systemic emergence of neutralizing antibodies [8] proteolytic degradation of circulating L-asparaginase by the proteases of the host organism is also responsible for the shorter half-life.Therefore, researchers working in the field of L-asparaginase focus on ways to circumvent these side effects along with techniques to improve its half-life *in-vivo*. The most popular approach, which has yielded satisfactory results, has been the chemical modification of asparaginase by covalently bonding the enzyme with polyethylene glycol or poly-(D, L-alanine) or dextran. The current standard-of-care formulation is L-asparaginase, obtained from *E. coli*, [9] covalently conjugated to polyethylene glycol (PEG) for extended circulation half-life and protection from immune responses. Thermal and pH stability, resist proteases and decrease the side effects caused by the release of a native

enzyme in systemic circulation. L-asparaginase nano formulations with liposomes , poly(d,l-lactide-co-glycolide) and hydrogel-magnetic nanoparticles are much focused on cancer research [10] .

MATERIAL AND METHODS

Preliminary screening by Plate Assay[11]

The actinomycetes isolated from soil samples subjected to plate assay to screen ISP4 medium (pH 6.8) with L aspargine and phenol red. Isolated Micromonospora sp is inoculated on the plate and incubated for 5-7 days. The pink zone around the colony indicates that the organism is able to produce L-asparaginase. The enzyme L-asparaginase will act on the substrate L-asparagine leading to production of ammonia which shifts the pH towards alkaline

Isolation of L asparaginase

100 ml M9 salt broth with aspargine monohydrate (0.2%) was prepared and autoclaved. Glucose used as carbon source and Phenol red used as indicator. 7 days old culture of *actino culture* ISP4 agar was taken and inoculated to the flask and kept under incubation for until the medium changed to pink. culture filtrate was mixed with 45% saturate ammonium sulphate and kept under refrigeration for 6 h and then subjected to vigorous shaking under stirrer. The sample was centrifuged and precipitate was collected and dialysed using dialysis membrane against phosphate buffer. Protein is redissolved in 2X sample buffer and separated by native PAGE. Followed by coomasie blue staining band were eluted from the gel using elution buffer.

Total protein estimation and enzyme assay[12]

The crude Protein content of the enzyme preparation was measured by Lowry's method. using bovine serum albumin as the standard. The concentration of protein during purification studies was calculated from the absorbance at 280nm. L-asparaginase activity was determined by measuring the amount of ammonia released by nesslerization according to the method described by Wriston and Yellin. The amount of ammonia liberated was calculated using ammonium (ammonium chloride) standard curve. One unit (U) of L-asparaginase is defined as the amount of enzyme which catalyzed the formation of 1 µmole of ammonia from L-asparagine per minute at 37 °C at pH 4-10.

Synthesis of Zinc oxide nanoparticles

100 mL of 5mM zinc sulphate was prepared in double distilled water and dissolved completely. 99 ml of zinc solution was mixed with 1 of protein(1mg/mL) and kept under shaking until the formation of precipitate. 1N NaOH was added to enhance precipitation. ZnNp was collected by centrifugation at 10,000 rpm for 30 min and subjected for UV analysis and characterized by SEM EDAX. Infrared spectra of L asparginase and ZnONp were obtained on a Bomen FT IR MB-102 spectrometer in KBr pellets to confirm protein

Anticancer study[13] human hepatocellular carcinoma (HepGII) was purchased from pune national cell line Laboratory and . Cells were maintained DMEM medium (Gibco, Gaithersburg, MD) supplemented with 10 % of fetal bovine serum (FBS: Gibco), 2 % of Pencillin-Streptomycin antibiotics (Gibco). Cultures were kept at 37° C in a humidified atmosphere of 95 % air and 5 % CO_2. 10 to 100µg/mL of L asparginase and ZnO conjugated L.asparginase were prepared on cell culture plate. 2 ×10^4 cells/well in tissue culture plates and incubated at 37°C, 5% CO_2. 25 µl of (0.5 mg/ml) MTT stain was added to each well, and the plates were incubated at 37°C for 4 h. 100 µl of dimethyl sulfoxide (DMSO) stop solution was added to each well. The plates were shaken at room temperature for 30 min. parthenolide used as positive control silver nitrate used as negative control. The plates were then read using ELISA Microplate reader at 570 nm. The percentage of viable cells was calculated. The half maximal inhibitory concentration (IC50) was calculated by fitting the survival curve using GraphPad Prism software in corporate

IC_{50} = (Control OD -Test OD/ Control OD) × 100%.

RESULTS AND DISCUSSION

M9 agar media supplemented with 0.09% phenol red to confirm their growth and production of L-asparaginase by Micromonospora sp. The production of L-asparaginase confirmed by color change due to a change in pH of the medium due to The release of ammonia during lysis of aspargine by Micromonospora sp. M9 medium used to produce L-asparaginase enzyme by submerged fermentation. L-asparaginase was purified from the crude enzyme using ammonium sulfate precipitation and dialysis. The total protein from M9 medium was estimated as 46 mg/mL. The enzyme activities were 26.24 U/ml±0.002. Cell viability results of L-asparaginase enzyme obtained from Micromonospora sp., the molecular weight and purity of the L-asparaginase enzyme was assessed by native PAGE. Protein analysis reveals presence of 3 different band with molecular weight of 32, 70 260 kDa. Among the three fraction L asparginase activity was retained in 70 kDa. The fraction was eluted and used for reduction of silver nanoparticle. 5mM Zinc sulphate is reduced under room temperature under

constant mixing by 15 min and confirmed by the excitation of maximum UV absorption at 460 nm and formation of yellow precipitate. Formation of strong broad absorption peak between 425nm and 475 nm, corresponding to the surface plasmon resonance (SPR) of the silver nanoparticles[14] . Terrestrial and marine Micromonospora spp has been less explored and have been successfully used biosynthesis of Silver nanoparticles.

FTIR (fig 2-3) of both ZnONp and asparginase shows presence of amide nitrogen stretching. Figure 2 shows the amide linkages between the amino acid residues in proteins observed at 3434 cm−1, assigned to the stretching vibrations of the primary amines, while the corresponding bending vibrations were seen at 1640 cm^{-1} C=C stretching, alkene in nature. Figure 3 crude asparaginase shows primary amine N=H stretching at 3449 cm^{-1} and N=H bending at 1640 cm^{-1}. Presence of free amine groups derived form cysteine residues in the protein already reported in many studies [15]. Similar amide vibration stretching was correlated with the work of Ritika et al. [16]. The SEM EDAX (fig 4) confirms formation of pure monodispersed, 35 to 70 nm spherical nano particle.

Viability of cell line treated with partially purified L-asparaginase extracted from Streptomyces sp have shown increased apoptosis by increase of enzyme concentration. The IC_{50} was recorded at 100µg/mL and 20% viability was recorded at 200 µg/mL. Whereas ZnO Np capped asparaginase showed IC_{50} at 60 µg/mL and 95% of cell inhibited at 100 µg/mL. The incubation of HepG2 cell line under different concentration of L-asparaginase enzyme showed gradual inhibition of cell growth as observed from its low IC_{50} value in Np conjugated than alone(fig 5-6). Further heamolytic assay of both asparaginase revals that they do not have cytotoxic effect. Asparaginase isolated by Prista and Kyridio [17] from *E. coli* had molecular weight of 33 kDa similarly Jain et al [18] reported 56kDa. L-asparaginase activity was studied as a function of pH in range between 4–10. The enzyme activity at pH 4 was 0.8 U increase gradually till pH 8 with maximum activity 26 U further decline at pH 9 as 6.8U. At acidic pH enzyme activity is ceased and higher pH's, enzyme activity was decreased. L-asparaginase, purified from Streptomyces sp exhibited optimum activity at pH 7.0 recorded as 22.8 U. These data results reveals maximal L-asparaginase activity of Streptomyces sp. was between pH 7-8.0 coincide with that report of Dhevagi and Poorani [19]. Baskar et al. [20] in 2015 proved that the synthesized nanobiocomposites of zinc oxide conjugated L-asparaginase has good anti-cancerous activity.

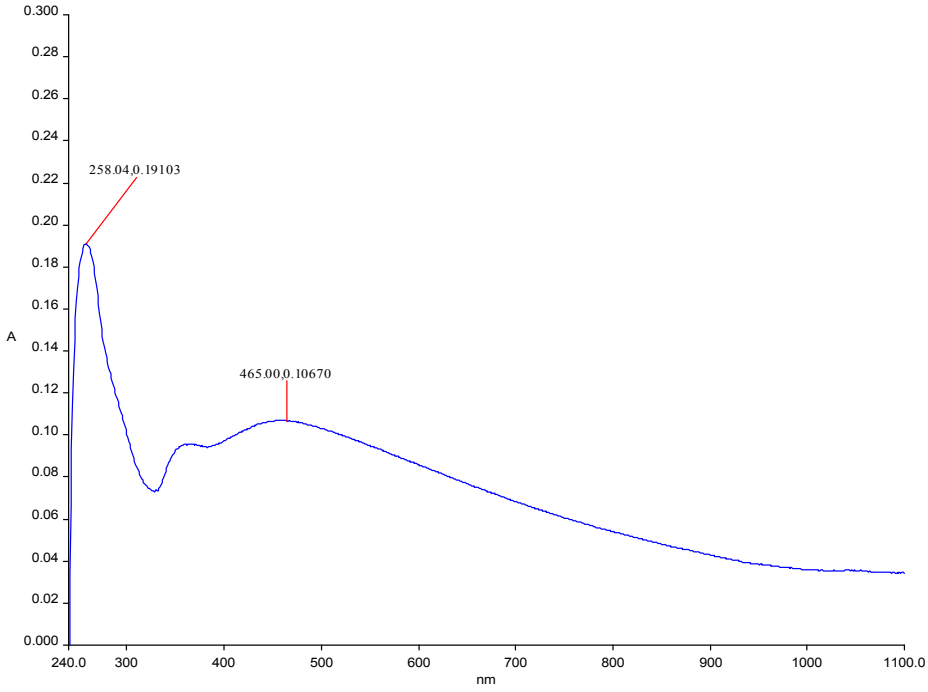

Figure 1.UV spectrum of Reduction of zinc sulphate

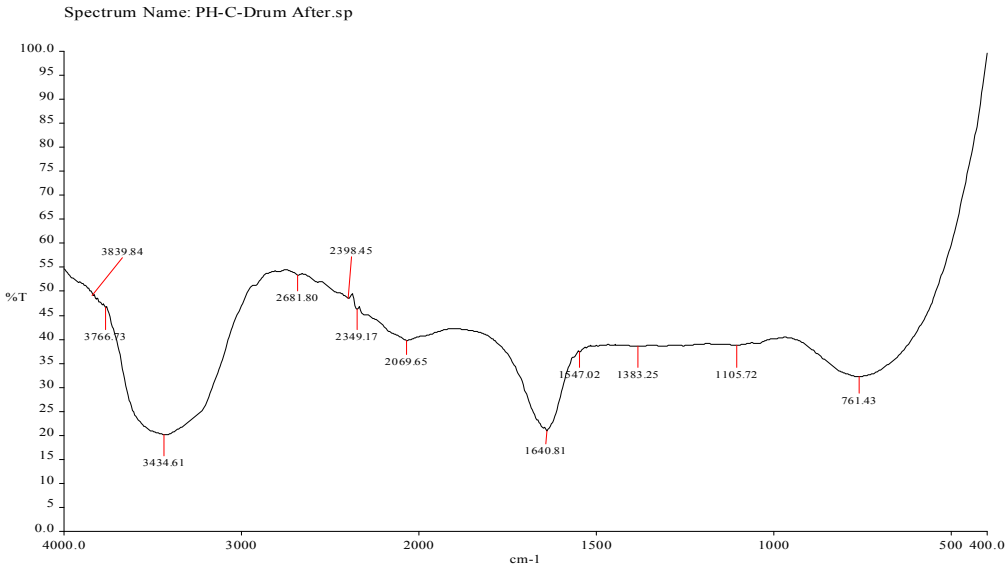

Figure 2.FTIR spectrum of ZnO nanoparticle reduced by L asparginase

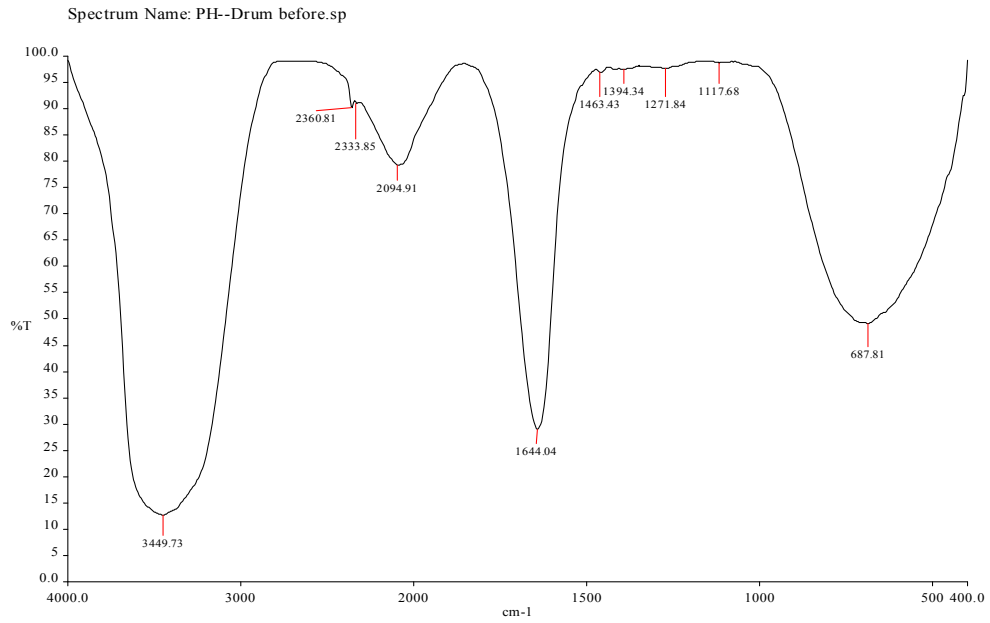

Figure 3. FTIR spectrum of Purified L asparginase

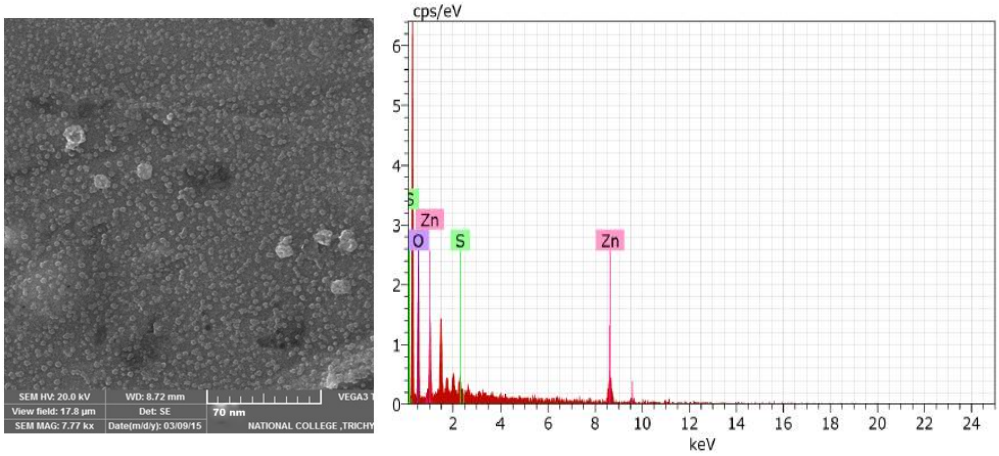

Figure 4. SEM AND EDAX of zinc oxide nanoparticles

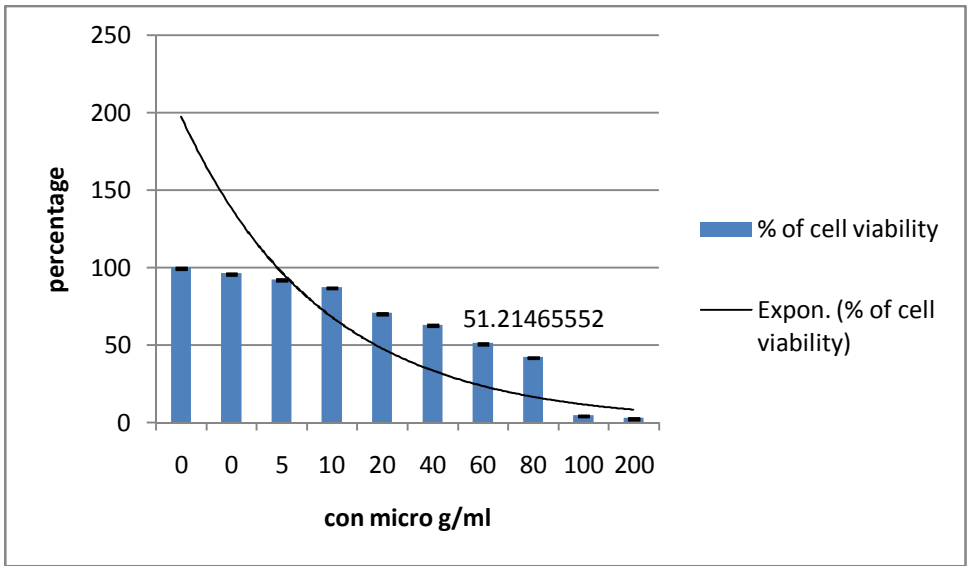

Figure 5.viability of cell treated with zno capped enzyme

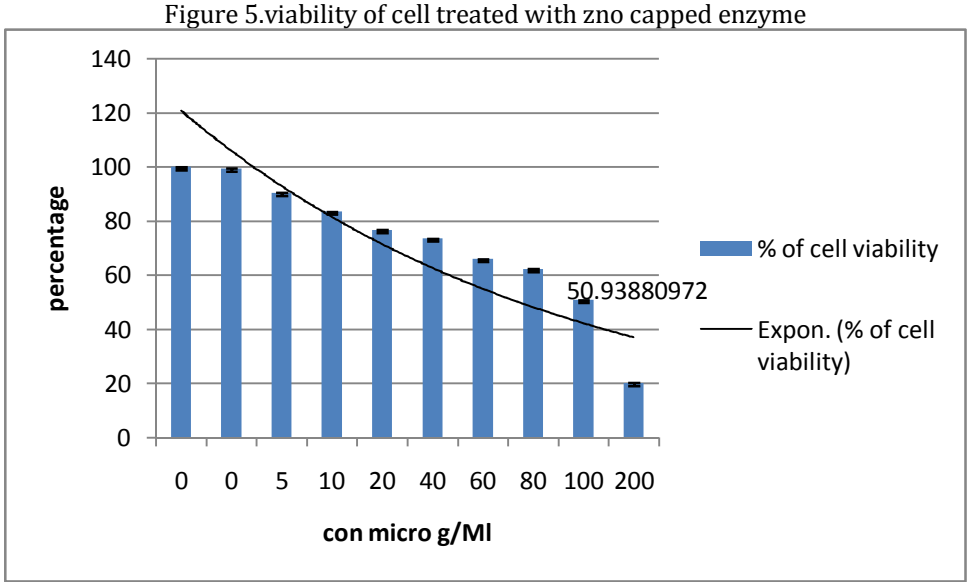

Figure 6.viability of cell treated with crude enzyme

CONCLUSION

L-asparaginase isolated from *Micromonospora sp* a salt tolerant actinobacterium has proven as potent anti-proliferative enzyme succefully conjugated with zinc nanoparticles and shows enhanced anticancer activity might be a novel approach.

REFERENCES

1. Molinski, T.F., Dalisay, D. S., Lieven, S. L., Saludes, J.P. (2008). Drug development from marine natural products. Nat. Rev. Drug discov.8: 69-85.
2. Boisselier, E., Astruc, D. (2009). Gold nanoparticles in nanomedicine: preparation, imaging, diagnostics, therapies and toxicit. ChemSoc Rev.38:1759–1782.
3. Kim, Y., Lee, BG., Roh, Y. (2013). Microbial synthesis of silver nanoparticles. J Nanosci Nanotechnol. 13:3897–3900
4. Mukundan, D., Mohankumar, R., Vasanthakumari, R. (2017). Comparative study of synthesized silver and gold nanoparticles using leaves extract of *Bauhinia tomentosa* Linn. and their anticancer efficacy. Bull Mater Sci 40(2):335-44
5. Jiang, Pi., Jin, H., Liu, R., Song, B., Wu, Q et al (2013). Pathway of cytotoxicity induced by folic acid modified selenium nanoparticles in MCF-7 cells. Appl. Microbiol. Biotechnol. 97: 1051
6. Tang, S., Zheng, J. (2018). Antibacterial activity of silver nanoparticles: structural effects. Adv Healthcare Mater.7:1701503.
7. Asgary, V., Shoari, A., Baghbani-Arani, F., et al. (2016). Green synthesis and evaluation of silver nanoparticles as adjuvant in rabies veterinary vaccine.IJN.11:3597–3605

8. Bahreini, E., Aghaiypour, K., Abbasalipourkabir, R., Mokarram, A., Goodarzi, M., Saidijam, M. (2014). Preparation and nanoencapsulation of l-asparaginase ii in chitosan-tripolyphosphate nanoparticles and in vitro release study. Nanoscale Res. Lett. 9(1), 340
9. Jain, R., Zaidi, K.U., Verma, Y., Saxena, P. (2012). L -asparaginase: A promising enzyme for treatment of acute lymphoblastic leukiemia. People's J. Sci. Res. 5 (1): 29-35
10. Teodor E, Litescu S.C, Lazar V, Somoghi R. [2009] Hydrogel-magnetic nanoparticles with immobilized l-asparaginase for biomedical applications J. Mater. Sci. - Mater. Med., 20 (6): 1307-1314,
11. Narayana, KJ., Kumar, KG., Vijayalakshmi, M. [2008] L-asparaginase production by Streptomyces albidoflavus. *Indian J Microbiol* 48(3):331-336.
12. EL-Sayed, M., El-Sayed, S.T., Wafaa, G.S., Abeer, N.S., Shimaa, S. [2011] Purification, Characterization and Antitumor Activity of L-asparaginase from Chicken liver. J. Am. Sci 7: 439-449
13. Weyermann, J., Lochmann D, Zimmer A. [2005] A practical note on the use of cytotoxicity assays. Int J Pharm 88: 369–76.
14. Sadhasivam, S., Shanmugam, P., Yun, K. [2010] Biosynthesis of silver nanoparticles by *Streptomyces hygroscopicus* and antimicrobial activity against medically important pathogenic microorganisms. Colloids Surf B Biointerfaces. 81(1):358-62.
15. Mandal, S., Phadtare, S., Sastry, M. [2005] Interfacing biology with nanoparticles. Curr Appl Phys 5:118–127.
16. Ritika, C., Abhishek Kumar., Jayanthi, A. [2013] A Biological Approach to the Synthesis of Silver Nanoparticles with *Streptomyces* sp JAR1 and its Antimicrobial Activity Sci Pharm. 81(2): 607–621
17. Prista, A., Kyridio, DA. [2001] L-asparaginase of *Thermo thermophilus*: Purification, properties and identification of essential amino acids for catalytic activity. Mol Cell Biochem 216:93-101
18. Jain, R., Zaidi, KU., Verma, Y., Saxena, P. [2012] L-asparaginase: A promising enzyme for treatment of acute lymphoblastic leukiemia. Peoples J Sci Res,5:29-3
19. Dhevagi, P., Poorani, E. [2006] Isolation and characterization of L-asparaginase from marine actinomycetes. Ind J Biotechnol; 5: 514–520.
20. Baskar, G., Chandhuru, J., Sheraz Fahad, K., et al. (2015) Anticancer activity of fungal L-asparaginase conjugated with zinc oxide nanoparticles. Journal of Materials science. Materials in Medicine. 26(1):5380.

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