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Production, optimization, and anti-cancer activity of L-asparaginase of *Pleurotus ostreatus* under solid state fermentation

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Key words: *Pleurotus ostreatus*, L- Asparaginase, Solid state fermentation, Cancer cell line.

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Abstract

Agro by-products like Wheat straw, Rice straw, Rice brane, Sugar cane bagasse, saw dust, Maize straw, Cumin straw, Corn seeds, Soya bean husks and Cowpea straw that collected from local farms in Gharbia Governorate, Egypt was used as substrates for production of the medically important L-Asparaginase enzyme under solid state fermentation (SSF). An attempt is made in the present study to optimize the production of L-asparaginase production by *Pleurotus ostreatus* using wheat straw under solid state fermentation (SSF). The maximum specific activity of L-asparaginase was 3.9 unit/mg that was achieved with the following optimized fermentation parameters: incubation period (9 days), initial moisture content (1: 025), pH 6.0, 30°C, supplemented with Tween 80 as surfactant. L-asparaginase was partially purified with 80 % ammonium sulfate saturation and used to test its efficiency against different cancer cell lines. L-asparaginase enzyme performed an Inhibitory activity against colon carcinoma cells, breast carcinoma cells, and mouse myelogenous leukemia carcinoma cells under an experimental conditions where it performed IC50 of 111 ± 5.4, 204 ± 9.8 and 390 ± 16.6 µg/ml respectively. We are working for the large-scale production of L- asparaginase using cheap growing sources through SSF.

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Introduction

L-asparaginase (E.C.3.5.1.1) belongs to an amidase group enzyme that hydrolyses the amide bond in L-asparaginase to L-aspartic acid and ammonia (Kumar and Verma 2012). L-asparaginase was produced throughout the world by submerged fermentation (SF) (Albanese and Kafkewitz 1978; Mostafa and Salama 1979; Radcliffe et al.1979). Solid state fermentation (SSF), is an exceptionally compelling method as the yield of L-asparaginase is commonly higher than that in SF (Arima1964). Additionally, SSF offers numerous other focal points including protection from defilement, simplicity of item extraction does not require entangled strategies for treating the matured residue (Lonsan et al.1985). Both of SF and SSF offers better open door for the biosynthesis of low-volume-high cost products (Balakrishnan and Pandey 1996). Screening and assessment of nutrition and natural prerequisites of microorganism is an imperative advance for bioprocess improvement. In current studies focus has done to series of agriculture bio-wastes like wheat, rice, maize, cowpea, cumin straws, rice brane, sugar cane bagasse saw dust, corn seed and soybean husks as cheap nutritional sources for growing *P. ostreatus* and examining its efficiency to form L-asparaginase as a valuable product.

To the best of our knowledge, this is the first report of generation of L-asparaginase in SSF using *P. ostreatus*. SSF was completed through a stepwise advancement methodology including, explanation of medium and ecological parts that influence compound creation essentially utilizing a one-factor at any given moment approach.

Chemotherapy is one of the important methods of treatment for malignant growth. It is the way toward utilizing anticancer medications to prevent cells from separating and duplicating however there are couple of healing chemotherapeutic medications against malignant growth because of high poisonous quality, non-selectivity, various symptoms, and low movement (Upm-Makna 2010). In this manner, seeking and creating compelling anticancer specialists for these specific ailments greatly affect grimness and mortality of the patients and could subsequently spare numerous lives. Relatively 60% of medication affirmed for disease treatment are of normal source.

The point of this investigation is to detach, distinguish and assess the capability of culturable *P. ostreatus* for generation of L-asparaginase as intense anticancer operators and build up a monetarily feasible bioprocess for generation of L-asparaginase by assessing and enhancing process parameters through controlling the healthful and physical parameters utilizing ease substrates.

Materials and methods

Microorganism

The culture of *P. ostreatus* was obtained from Agricultural Research Center, Mushroom Laboratory culture collection (MLCC), Egypt. It was maintained on Potato Dextrose Agar (PDA) slants.

Evaluation of L-asparaginase production by plate assay

The fungus was evaluated for its ability to produce L-asparaginase by rapid plate assay based on (Imada et al, 1973). Modified Czapek Dox, medium contains L-asparagine as the sole nitrogen source, and it was supplemented with Phenol red (0.005 g/L) as pH indicator, pH was adjusted to 6.5.

The media was autoclaved, and plates were inoculated and incubated for 72 hours. Two control plates were prepared, uninoculated medium served as negative control, Sodium nitrate was used in the medium instead of asparagine, and organism was inoculated served as positive control.

Substrates for SSF

Agro by-products were collected from local farms in Gharbia Governorate, Egypt. Ten agro by-products were used (Wheat straw, Rice straw, Rice brane, Sugar cane bagasse, saw dust, Maize straw, Cumin straw, Corn seeds, Soya bean husks and Cowpea straw), All the substrates used in the study were procured from the local market.

Five grams of each substrate separately was taken in 100 ml Erlenmeyer flask and rehydrated to 50% of moisture content by adding sterilized L-asparaginase medium and a parallel set of flasks with sterilized distilled water (Nair et al. 2013).

The flasks were aseptically inoculated with 10 mycelial plugs (7 mm) of 7-day-old culture and incubated at 30°C for a period of 12 days, Enzymes were extracted by adding 25 mL of phosphate buffer (pH 7.0; 100 mM) to each culture flask and kept on a (180 rpm) at 30°C for 1 hour. The mixtures were filtered through a sterile cotton cloth and the filtrate obtained was centrifuged (REMI C-24 BL) at 5,000 rpm at 4°C for 20 min. The supernatant obtained was analyzed for enzyme activities and extracellular proteins (Usha, 2014)

Extraction of L-asparaginase

The samples were withdrawn under aseptic condition. 1gm each of moldy substrate was taken into a beaker and distilled water (1:10) was added to it. Proper mixing was done by using vortexing for 15 min in the presence of 20µL tween 60. The extract was filtered using Wattman filter No.1 followed by centrifugation at 4000 rpm for 10 min. Supernatants obtained were used as crude enzyme preparations and the same were used for the assay.

Enzyme Assay

L-asparaginase activity was determined by nesslerization (Imada et al., 1973) where the rate of hydrolysis of L-asparagine is determined by measuring the released ammonia. All reaction mixtures were measured separately by micro plate spectrophotometer reader at 450 nm in triplicate.0.5ml of 0.01M L-asparagine was taken in a test tube, to which 0.5ml of Tris-HCl Buffer (0.05 M and pH 8.6), 0.5ml of enzyme and 0.5ml of distilled water was added to make up the volume up to 2.0 ml and the reaction mixture was incubated for 30 min. After the incubation period the reaction was stopped by adding 0.5ml of 1.5M Trichloroacetic acid (TCA). From the above reaction mixture 0.1ml was taken and added to 3.7ml distilled water and then 0.2ml of Nessler's reagent was added and incubated for 15 to 20 min at 37°C. The optical density (OD) was measured at 450 nm (Labomed, INC Spectrophotometer).

The blank was run by adding enzyme preparation after the addition of TCA. The enzyme activity was expressed in International unit. One IU of L-asparaginase activity was defined as that amount of enzyme which catalyze the formation of 1µmole of NH3 per minute per ml.

Wheat straw was the best source of asparaginase enzyme synthesis and was therefore selected for the following experiments

Enzyme partial purification

L-asparaginase enzyme was extracted from the fermented substrate and purified by salting out with finally grinded ammonium sulphate fractionation. The maximum enzyme activity was obtained with 80% ammonium sulfate precipitation which was dialyzed kept for cytotoxic activity test.

Optimization of process parameters to produce L-asparaginase

Various fermentation parameters that improve the yield of L-asparaginase by *P. osteratus* under solid state fermentation were investigated. The effect of incubation time (2 -9 days), temperature (25°C-40°C), initial moisture content of the substrate (1:01 - 1:05), initial pH (3-8, adjusted with 1N HCl or 1N NaOH), inoculum age (2-11 days) were evaluated (Vijay and Jaya Raju, 2015). The effect of five surfactants (Tween 20, Tween 80, Triton-X, Ethylene Diamine Tetra Acetate (EDTA) sodium dodecyl sulfate (SDS)) on production of L-asparaginase were studied

Cytotoxicity of L-asparaginase enzyme

It was tested against three Mammalian cell lines according to Mosmann (1983) and Gomha et al. (2015): MCF-7 cells (human breast cancer cell line, HepG-2 cells (human Hepatocellular carcinoma) and MNFS-60 (Mouse Myelogenous Leukemia

carcinoma) were obtained from VACSERA Tissue Culture Unit.

Cell line Propagation

The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50µg/ml gentamycin. All cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were subcultured two times a week.

Cytotoxicity evaluation using viability assay

For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1×10^4 cells per well in 100µl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37°C in a humidified incubator with 5% CO_2 for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO.

The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for at 37°C, various concentrations of sample were added, and the incubation was continued for 24 h and viable cells yield was determined by a colorimetric method.

In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed, and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated**.** The optical density was measured with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [(ODt/ODc)] x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graphpad Prism software (San Diego, CA. USA)

Results

Effect of different substrates on L- asparaginase activity of P. ostreatus

The results in table (1) indicated that Wheat straw was the most effective substrate for L-asparaginase activity (6.44 U/ml) followed by Saw dust and Maize straw where the activity of L- asparaginase were 5.06 and 4.52 respectively. The other carbon sources also showed L-asparaginase activity but the yield was decreased with respect to Rice brane, Cowpea straw. However, the lowest L-asparaginase activity was 0.23 U/ml was obtained with using Soya bean husks. Statistical analysis showed very highly significant effects of different carbon sources on L- asparaginase activity of *P. ostreatus* at P value ≤ 0.001.

Effect of different incubation periods on L-asparaginase activity of P. ostreatus

The results presented in Fig (1) showed that the highest L-asparaginase activity (6.4 U/ml) was recorded at the end of 9 days of incubation period. The results also showed that L-asparaginase activity was decreased gradually with further extension of the incubation periods to 7 days. Statistical analysis explained very highly significant values for dry weight

and L-asparaginase activity of *P. ostreatus* at P ≤ 0.04 under the effect of different incubation periods.

Effect of different incubation temperatures on L-asparaginase activity of P. ostreatus.

The results on Fig (2) showed that the dry weight and L-asparaginase activity of *P. ostreatus* increased with increasing incubation temperature where the maximum L-asparaginase activity was 11.01U/ml at 30°C. Above and below this degree of temperature, the L-asparaginase activity of *P. ostreatus* was substantially lower (Fig. 2).

Anova test obvious very highly significant values for L-asparaginase activity of *P. ostreatus* at P ≤ 0.001 under the effect of different incubation temperatures.

Table 1. Effect of different substrates on L- asparaginase activity of *P. ostreatus*

Substrate	L-asparaginase activity (U/ml)
Wheat straw	6.44
Rice straw	3.70
Rice brane	2.10
Sugar cane bagases	3.19
Saw dust	5.06
Maize straw	4.52
Cumin straw	0.73
Corn seeds	2.92
Soya bean husks	0.23
Cowpea straw	2.39

Effect of different moisture content on L-asparaginase activity of P. ostreatus

The evaluation of the initial Moisture content of substrate indicated that 1:.025 ratio is the best for *P. ostreatus* growth under solid state fermentation (Fig.3).

Effect of different pH values on L- asparaginase activity of P. ostreatus

The results represented in figure (4) revealed that pH 6.0 was the optimal pH for L-asparaginase activity (7.27 U/ml). Above and below pH 7 the activity of L-asparaginase of *P. ostreatus* was substantially lower.

Table 2. Effect of surfactants on L- asparaginase activity of P. *ostreatus*.

Surfactants	L-asparaginase activity (U/ml)
Tween 20	6.92±0.0216
Tween 80	8.36±0.0355
Triton-X	7.66±0.0326
Ethylene Diamine Tetra Acetate (EDTA)	5.17±0.0216
Sodium dodecyl sulfate (SDS)	4.59±0.0216

The experimental fungus has the ability to grow and produce extracellular L-asparaginase at a wide range of pH values (3, 4, 5, 6 ,7and 8). Statistical analysis showed very highly significant values L-asparaginase activity of *P. ostreatus* at P value ≤ 0.001 under the effect of different pH values.

Effect of different inoculum age on L- asparaginase activity of P. ostreatus

The results in Fig. (5) showed that the age of inoculum exerted a significant effect on L-asparaginase activity. The highest L-asparaginase activity (7.89 U/ml) was on the ninth day.

Effect of surfactants on L- asparaginase activity of P. ostreatus

This experiment was carried out to evaluate the effect of some surfactants on L-asparaginase activity of *P. ostreatus*. L-asparaginase activity of *P. ostreatus* was enhanced by the presence of Tween 80 (8.36 U/ml) followed by Triton-X and Tween 20 (7.66 and 6.92 U/ml) respectively.

Table 3. Purification of L-asparaginase enzyme from *P. ostreatus* using SSF.

Purification steps	Total activity (U*)	Total protein (mg)	Specific activity (U/mg)	Purification fold	Recovery yield %
Crude L-asparaginase	4180	1070	3.906	1	100
Ammonium sulphate precipitation	1914	186	10.29	2.634	45.798
Dialysis	874.6	24	36.44	9.32	20.92

*One IU of L-asparaginase activity was defined as that amount of enzyme which catalyzes the formation of 1µmole of NH_3 per minute per ml.

The lowest activity was recorded with sodium dodecyl sulfate (SDS).

Purification of L-asparaginase enzyme from P. ostreatus using SSF

L-asparaginase enzyme was extracted from the fermented substrate and purified by salting out with ammonium sulphate and dialysis. The purification steps were illustrated in table (3) showed that the first step of the purification by ammonium sulfate precipitation (80%) was achieved 2.63 purification fold with a specific activity of 10.29 U/mg and recovery yield percentage of 45.798 %.

The second purification step was done using dialysis, this step showed 9.32 purification fold with a specific activity of 36.44 U/mg and recovery yield percentage of 20.92 %

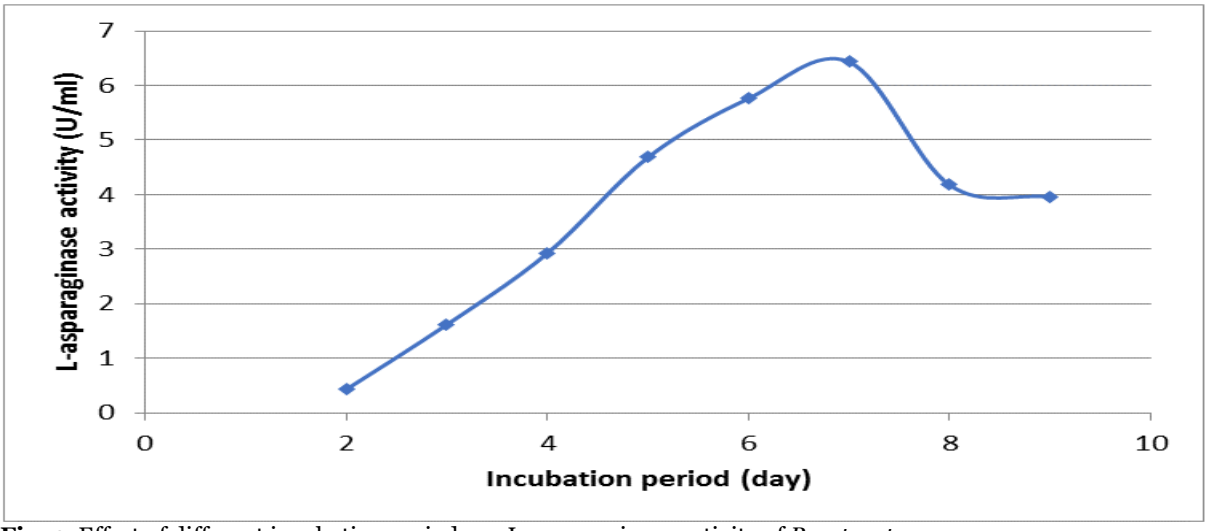

Fig. 1. Effect of different incubation periods on L- asparaginase activity of *P. ostreatus*.

Cytotoxicity evaluation

L-asparaginase enzyme performed a Inhibitory activity against colon carcinoma cells, breast carcinoma cells, and mouse myelogenous leukemia carcinoma cells under these experimental conditions with IC50 = 111 ± 5.4, 204 ± 9.8 and 390 ± 16.6 µg/ml respectively (Fig 6 A, B and C).

Discussion

Solid state fermentation has developed as a potential innovation for the generation of microbial products using the inexpensively accessible crude materials. The results in table (1) indicated that wheat straw was the most effective substrate for L-asparaginase activity (6.44 U/ml) followed by Saw dust and Maize straw where the activity of L- asparaginase were 5.06 and 4.52 U/ml, respectively. However many literatures have listed that Soya bean as substrate is super for L-asparaginase generation (Abdel-Fattah and Olama, 2002; El-Bessoumy et al. 2004), also squanders has listed from three leguminous yields grain of *Cajanus cajan*, *Phaseolus mungo* and *Glycine max* in light of simplicity of cleaning and ease of common materials and for the business generation

of the catalyst, choice of a predominant strain and substrate is a vital advance (Mishra, 2006). Additionally, the utilization of copiously accessible modest agro-modern waste like sesame oil cake which is a perfect wellspring of proteinaceous supplements with an unrefined protein substance of 35.6% would be in a perfect world suited supplement bolster in SSF rendering both carbon and nitrogen sources and is accounted for to be a decent substrate for L-asparaginase creation (Kuo, 1967).

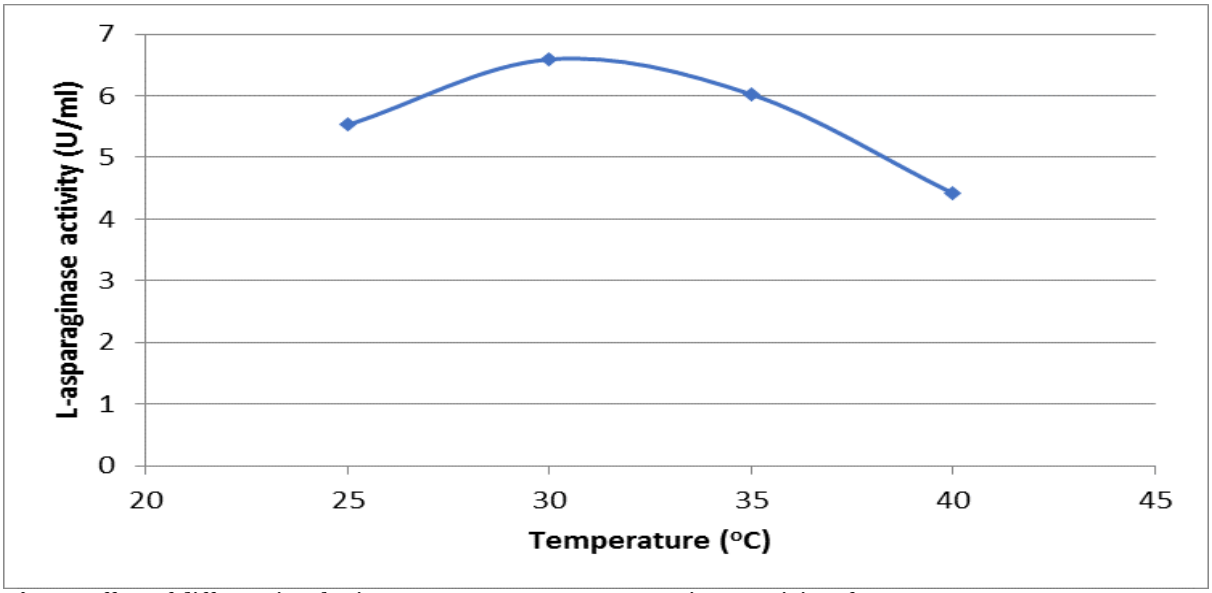

Fig. 2. Effect of different incubation temperatures on L- asparaginase activity of *P. ostreatus*.

The results presented in Fig (1) showed that the highest L-asparaginase activity (6.4 U/ml) was recorded at the end of 7 days of incubation period. The results also showed that L-asparaginase activity was decreased gradually with further extension of the incubation periods to 9 days. Hosamani and Kaliwal (2011) mentioned that the creation of L-asparaginase began at 24 hours and achieved most extreme at 48 hours and the diminished fundamentally with increment in the incubation time. Similar results have been reported by Lapmak et al. (2010) where the highest activity of 6.3 U/ml for 72 hours using *Bipolaris* sp.BR438 where as Venil and Lakshmanaperumaisamy (2009) have reported maximum L-asparaginase production of 79.84 U/gds at 36 hours of incubation. At longer incubation periods, the chemical action diminished which may be because of the exhaustion of supplements, collection of poisonous end.

Chanakya et al. (2011) reported that the improvement of inoculum volume is important as high inoculum levels are inhibitory in nature. In this study, L-asparaginase creation expanded promptly with the expansion in the inoculum level and most extreme protein action was gotten at 20% (v/w) whereas the slightest action was acquired at half (v/w) inoculum level.

Chanakya et al. (2011) have detailed the most extreme L-asparaginase generation of 4.81 IU with the inoculum volume of 1.5 ml of 7 days old *Fusarium oxysporum*. The present outcomes were in great concurrence with Sreenivasulu et al. (2009) who have detailed 50.2 U/gds with 20% (v/w) inoculum level.

The reason might be a result of higher inoculum thickness is inhibitory to the protein generation as an excessive amount of biomass can drain the substrate supplements or gathering of some non-unpredictable self-repressing substances that restrains the item development (Bilgrami and Verma 1981) and bring down thickness may give inadequate biomass causing incited item arrangement whereas higher inoculum may deliver excessively biomass which is inhibitory to the item development (Mudgetti 1986).

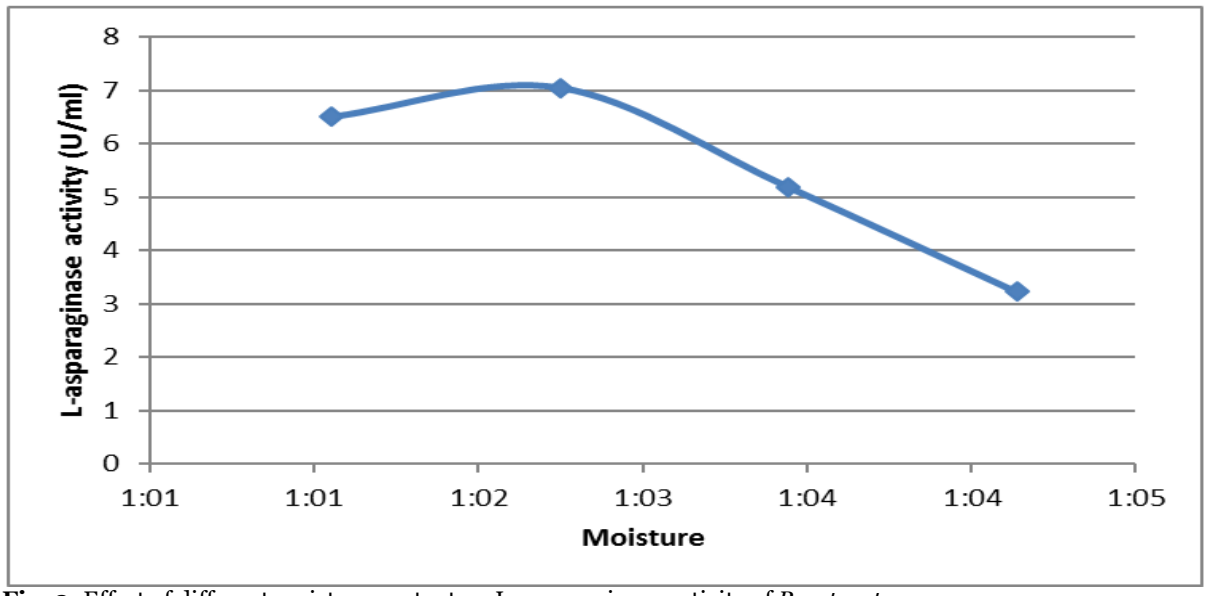

Fig. 3. Effect of different moisture content on L- asparaginase activity of *P. ostreatus*.

The results on Fig (2) showed that the dry weight and L-asparaginase activity of *P. ostreatus* increased with increasing incubation temperature where the maximum L-asparaginase activity was 11.01U/ml at 30°C. *Fusarium culmorum* and *F. brachygibbosum*. showed maximum activity of L-asparaginase at 30°C, as reported by earlier workers in *Mucor hiemalis* (Monica et al.2013), *Penicillium* sp. (Kotra et al.2013), *Aspergillus terreus (*Balasubramanian et al. 2012) and *Emericella nidulans* (Jayaramu et al.2010). It is obvious that fungi being mesophilic in nature prefer to grow with high metabolic rate at this temperature. But, on the contrary 35°C was the optimum temperature for the activity of L-asparaginase was reported in Penicillium sp. (Mohsin et al.2012).

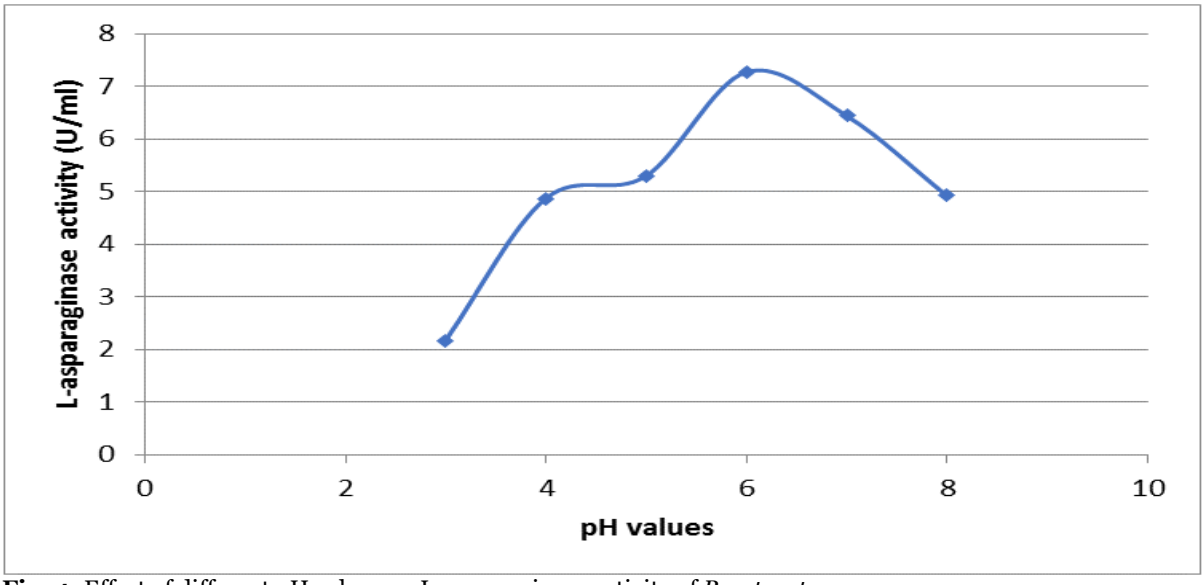

Fig. 4. Effect of different pH values on L- asparaginase activity of *P. ostreatus*.

The results in Fig. (5) showed that the age of inoculum exerted a significant effect on L-asparaginase activity. The highest L-asparaginase activity (7.89 U/ml) was on the ninth day. L-asparaginase activity of *P. ostreatus* was enhanced by the presence of Tween 80 (8.36 U/ml) followed by Triton-X and Tween 20 (7.66 and 6.92 U/ml) respectively. The lowest activity was recorded with

sodium dodecyl sulfate (SDS).

Table (3) showed that the first step of the purification by ammonium sulfate precipitation (80%) was achieved 2.63 purification fold with a specific activity of 10.29 U/mg and recovery yield percentage of 45.798 %. The second purification step was done using dialysis, this step showed 9.32 purification fold with a specific activity of 36.44 U/mg and recovery yield percentage of 20.92 %. L-asparaginase from various fungal species have been purified and characterized and reported earlier. L-asparaginase from *Penicillium brevicompactum* NRC 829 was purified to 151.12-fold with a specific activity of 574.24 U/mg and yield of 39.90% (Elshafei et al.2012) and L-asparaginase purified from *Mucor hiemalis* exhibited a specific activity 69 U/mg with 18.46% recovery and 4.59 purification fold (Monica et al.2013).

Fig. 5. Effect of different inoculum age (day) on L- asparaginase activity of *P. ostreatus*.

McAllister et al. (2000) reported that Tween 80 increased the stability and substrate binding capacity of enzymes under in vitro conditions. One of the most important limitation of L-asparaginase as anticancer drug is that the allergic reactions exhibited by immune system of the patients receiving the medication of L-asparaginase. The patient immune system reacts in many different ways against the drug such as, the development of high titers of serum IgG antibodies which in much of cases interfere with the therapeutic effect of the enzyme (Usman 2015). Drug's immunogenicity is fundamental obstacle which limits the therapy with foreign proteins in humans. A real immunological tolerance that would require antigen specific T-cell mediated immunosuppression is difficult to achieve. One way to overcome this problem for a limited time is to switch to another preparation (Neeta Asthana and Azmi 2003). The chemotherapeutic operators in random incorporate restoratively compounds as L-asparaginase that follow up on explicit cell receptors to upsets DNA in the lion's share of tumor cells (McKnight et al.2003).

The basis behind L-asparaginase relies on the way that tumor cells are lacking in aspartate smelling salts ligase action which limits their capacity to blend the regularly superfluous amino corrosive L-asparagine. These leukemic cells rely upon coursing L-asparagine.

The activity of the L-asparaginase does not influenced the working of typical cells which can blend enough for their very own necessities however decrease the free exogenous fixation thus incites a condition of deadly starvation in the defenseless tumor cells. Alongside strain enhancement by improvement of creation parameters, the yield of L-asparaginase profitability was augmented.

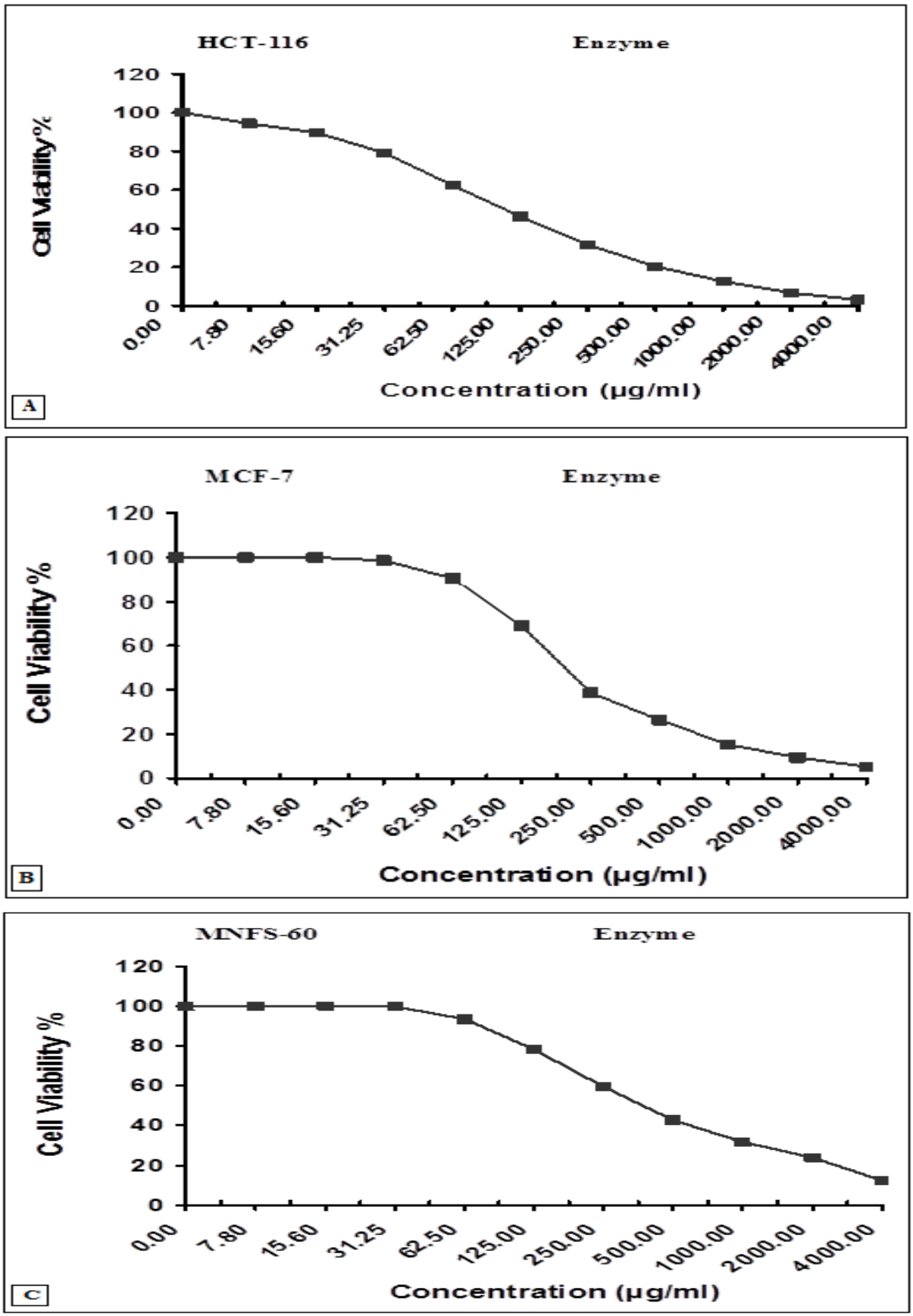

Fig. 6. Evaluation of L-asparaginase enzyme cytotoxicity against Mammalian cell lines: MCF-7 cells (human breast cancer cell line (A), HepG-2 cells (human Hepatocellular carcinoma) (B)and MNFS-60 (Mouse Myelogenous Leukemia carcinoma) were obtained from VACSERA Tissue Culture Unit (C).

Conclusion

From this study, it is clearly indicated that the tested agriculture by products can provide a rich source for growing P. ostreatus as a source for the medically important L-asparaginase.

L-asparaginase enzyme performed an Inhibitory activity against colon carcinoma cells, breast carcinoma cells, and mouse myelogenous leukemia carcinoma cells. However, more detail investigation is required to completely purify this microbial enzyme, which may be effectively used in the large-scale production using cheap growing sources through SSF for commercial and pharmaceutical purposes in the future.

References

Abdel-Fattah YR, Olama Z. 2002. L- asparaginase production by Pseudomonas aeruginosa in solid-state culture: evaluation and optimization of culture conditions using factorial designs. Process Biochemistry **38(1),** 115-122.

Albanese E, Kafkewitz K. 1978. Effect of medium composition on the growth and asparaginase production of vibrio succinogenes. Applied Environmental Microbiology **36,** 25-30

Arima K. 1964. Microbial Enzyme Production. In: Global Impacts of Applied Microbiology, Starr, M.P. (Ed.). John Wiley and Sons, New York, p 279-294.

Balakrishnan K, Pandey A. 1996. Production of biologically active secondary metabolites in solid state fermentation. Journal of Scientific and Industrial Research **55,** 365/72.

Balasubramanian K, Ambikapathy V, Panneerselvam A. 2012. Production, isolation, and purification of L-asparaginase from Aspergillus terreus using submerged fermentation. International Journal of Advances in Pharmaceutical Research **3(2),** 778 – 783.

Bilgrami KS, Verma RN. 1981. 2nd ed, Vikas Publishing, Pvt. Ltd, 313-315.

Chanakya P, Nagarjun V, Srikanth M. 2011. International Journal of Pharmaceutical Sciences Review and Research **7(2),** 189-192.

El-Bessoumy AA, Sarhan M, Manjour J. 2004. Production, isolation and purification of L-asparaginase from Pseudomonas aeruginosa 50071 using solid- state fermentation. Journal of biochemistry and Molcular biology **37(4),** 387-393.

Elshafei AM, Hassan MM, AbouZeid MA, Mahmoud DA, Elghonemy DH (2012) Purification, characterization and antitumor activity of L-asparaginase from Penicillium brevicompactum NRC 829. British Microbiology Research **2(3),** 158-174.

Gomha SM, Riyadh SM, Mahmmoud EA, Elaasser MM. 2015. Synthesis and Anticancer Activities of Thiazoles, 1,3-Thiazines, and Thiazolidine Using Chitosan-Grafted-Poly(vinylpyridine) as Basic Catalyst. Heterocycles **91(6),** 1227-1243.

Hosamani R, Kaliwal BB. 2011. L-asparaginase-an anti-tumor agent production by fusarium equiseti using solid state fermentation. International Journal of Drug **3(2),** 88-99.

Imada A, Igarasi S, Nakahama K, Isono M. 1973. Lasparaginase and glutaminase activities of microorganisms. Journal of General Microbiology **76,** 85-99.

Imada A, Igarasi S, Nakahama K, Isono M. 1973, Asparaginase and glutaminase activities of microorganisms. Journal of General Microbiology. **76,** 85-99.

Jayaramu M, Hemalatha NB, Rajeshwari KG, Siddalingeshwara KG, Mohsin SM, Sunil Dutt PLNSN. 2010. A novel approach for detection, confirmation and optimization of L-asparaginase

from Emericella nidulans. Current Pharmaceutical Research **1(1)**, 20-24.

Kotra SR, Prudvi N, Sada Sai KRA, Mannava KK, Peravali JB, Anmol Kumar, Sambasiva Rao KRS, Pulicherla KK. 2013. Cost effective process for the production of fungal L-asparaginases from Penicillium sps isolated from local soil sample. Mintage Journal of Pharmaceutical and Medical Sciences **2(1)**, 45-50.

Kumar K, Verma N. 2012. The various sources and application of L-asparaginase. Asian Journal of Biomedical and Pharmaceutical Sciences **3**, 197-205.

Kuo LH. 1967. Animal feeding stuffs compositional data of feeds and concentrates (Part 3). Journal of Malaysian Agriculture **46,** 63-70.

Lapmak K, Lumyong S, Thongkuntha S, Wongputtisin P, Sardsud U. 2010. Chiang Mai Journal of Science **37,** 160–164.

Lonsan BK, Ghildyal NP, Budiatman S, Ramakrishna SV. 1985. Engineering aspects of solid-state fermentation. Enzyme and Microbial Technology **7,** 258/65.

McAllister TA, Stanford K, Bae HD, Treacher R, Hristov AN, Baah J, Shelford JA, Cheng KJ. 2000. Effect of a surfactant and exogenous enzymes on digestibility of feed and on growth performance and carcass traits of lambs. Canadian journal of Animal science **80,** 35-44.

McKnight S. 2003. Gene switching by metabolic enzymes--how did you get on the invitation list? Cell **114,** 150-152.

Mishra A. 2006. Appl. Biochem. Biotechnol, **135,** 33-42.

Mohsin SM, Sunil Dutt PLNSN, Siddalingeshwara KG, Karthik J, Jayaramu M, Naveen M, Vishwanatha T, Prathiba KS. 2012. Optimization of fermentation conditions for the biosynthesis of L-Asparaginase by Penicillium sp. Journal of Academia and Industrial Research **1(4),** 180-182.

Monica T, Lincoln L, Niyonzima FN, Sunil SM. 2013. Isolation, purification and characterization of fungal extracellular L-asparaginase from Mucor Hiemalis. Journal of Biocatalysis and Biotransformation **2(2),** 1-9.

Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological. Methods **65,** 55-63.

Mostafa SA, Salama MS. 1979. L-asparaginase producing Streptomyces from soil of Kuwait. Zentralbl Bakteriol Naturwiss **134(4),** 325–334.

Mudgetti RE, In Demain AL, Soleman NA. 1986. American Society of

Nair A, Kumar R, Agalya Devi R. 2013. Screening of commonly available solid process residues as substrate for L-asparaginase production by Aspergillus terreus MTCC 1782. Research Journal of Pharmaceutical, Biological and Chemical Sciences **4,** 1731–1737.

Neeta Asthana S, Azmi W. 2003.Microbial L-asparginase: A potent antitumor enzyme. Indian Journal of Biotechnology **2**, 184–94.

Radcliffe CW, Kafkewitz D, Abuchowski A .1979. Asparaginase production by human clinical isolates of Vibrio succinogenes. Applied Environmental Microbiology **38,** 761-762.

Sreenivasulu V, Jayaveera KN, Mallikarjuna Rao P. 2009. Research Journal of Pharmacognosy and Phytochemistry **1,** 30-34.

Upm-Makna. 2010. Cancer research laboratory, Institute of Bioscience.

Usha KY, Praveen 1K, Reddy BR. 2014. Enhanced Production of Ligninolytic Enzymes by a Mushroom Stereum ostrea**.** Biotechnology Research International Article ID 815495, p 1-9.

Usman A. 2015. Seeking efficacy in L-asparaginase to combat acute lymphoblastic leukemia (ALL): a review. African Journal of Pharmcy and Pharmacology **9,** 793–805.

Venil C, Lakshmanaperumalasamy P. 2009. The Internet Journal of Microbiology **7(1),** 10-18.