

Full Length Research Paper

L-Asparaginase produced by *Streptomyces* strain isolated from Egyptian soil: Purification, characterization and evaluation of its anti-tumor

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L-Asparaginase (EC 3.5.1.1) is produced from actinomycetes to avoid the hypersensitive effect of that produced from other bacteria. *Streptomyces halstedii* strain was isolated from Egyptian soil and produced L-asparaginase. The 55.2-fold purified enzyme obtained had a final specific activity of 2071.2 U/mg. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) showed one band with molecular weight of 100 KDa. The K_m value was 0.1939 mM. The enzyme showed maximum activity at pH 8.0, at optimum temperature at 37°C. Ethylene diamine tetraacetic acid (EDTA) and metal ions such as Zn^{2+} , Hg^{2+} , Cu^{2+} and K^+ decreased the activity of the enzyme. Ions such as Ca^{2+} and Fe^{2+} did not affect the activity of the enzyme. The enzyme showed cytotoxic activity against Ehrlich ascites cells (EAC) *in vitro*. *In vivo*, it showed a significant reduction in malondialdehyde (MDA) levels as the end product of lipid peroxidation and a remarkable increase in activity of liver antioxidant enzymes, [superoxide dismutase (SOD), catalase (CAT)] and a reduction in tumor weight. In conclusion, L-asparaginase from *S. halstedii* showed anti-tumor activity and cytotoxic effect against cancer cell line *in vitro* and *in vivo*. The reduction of tumor size in albino mice may be attributed to the elevation of CAT and SOD activities as well as the diminishing of MDA.

Key words: L-Asparaginase purification, antitumor activity, *Streptomyces* sp.

INTRODUCTION

L-Asparaginase (EC 3.5.1.1) is the first enzyme with anti-tumor activity intensively studied in human beings (Savitri and Azmi, 2003). It is used for the treatment of malignancies of the multiorgans (Kumar and Selvam, 2011). It is used widely as a therapeutic agent for treating acute lymphoblastic leukemia in children and lymphosarcoma (Khamna et al., 2009).

The amino acid, L-asparagine, is essentially required for the survival of both normal and cancer cells. Most normal tissue synthesizes L-asparagine in amounts sufficient for their metabolic needs with their own enzyme, L-asparagine synthetase. However, certain malignant cells

cannot synthesize L-asparagine and must consequently rely on an external supply in the plasma and tissues. Since several types of tumor cells require L-asparagine for protein synthesis, they are deprived of an essential growth factor in the presence of L-asparaginase. The administration of this enzyme can digest its substrate resulting in starving and killing certain cancer cells (Basha et al., 2009; Kumar and Selvam, 2011). This fact suggested the development of this enzyme as a potent anti-tumor or anti-leukemic drug (Savitri and Azmi, 2003).

Since extraction of L-asparaginase from mammalian cells is difficult, microorganisms have proved to be a

better alternative for L-asparaginase extraction, thus facilitating its large scale production (Sahu et al., 2007). Various bacterial sources such as *Escherichia coli*, *Staphylococcus* sp., *Pseudomonas aeruginosa* (Moorthy et al., 2010), *Thermus thermophilus* (Pritsa et al., 2001), *T. aquaticus* (Curran et al., 1985), *Enterobacter aerogenes* (Mukherjee et al., 2000), *Zymomonas mobilis* (Pinheiro et al., 2001) and *Bacillus licheniformis* (Golden and Bernlohr 1985) produce L-asparaginase. However, L-asparaginase from bacterial sources causes hypersensitivity in the long-term use, leading to allergic reactions and anaphylaxis (Sahu et al., 2007).

The search for other L-asparaginase sources, like actinomycetes, can lead to an enzyme with less adverse effects. Actinomycetes represent a good source for the production of L-asparaginase (Savitri and Azmi, 2003). The production of L-asparaginase has been studied in *Streptomyces griseus*, *S. Karnatakensis*, *S. albidoflavus*, *S. gulbargensis* (Kattimani et al., 2009), *S. aurantiacus* (Gupta et al., 2007), *S. aureofasculus*, *S. canus*, *S. olivoviridis* (Sahu et al., 2007), a marine *Streptomyces* sp. PDK2 (Narayana et al., 2008) and *Nocardia* sp. (Gunasekaran et al., 1995).

This work aimed to purify, to characterize and to assess the potential anti-tumor activity of L-asparaginase from *S. halstedii* isolated from Egyptian soil.

MATERIALS AND METHODS

Isolation of actinomycetes

Soil samples were collected from different localities of Menoufiya governorate located in the Northern of Egypt. Thirty (30) isolates of actinomycetes were isolated from soil by the dilution plate method on starch-nitrate agar plates (Waksman and Lechevalier, 1962). The plates were incubated for seven days at 30°C. Colonies were checked for purity by repeated sub-culturing and the pure colonies maintained on slants of the same medium and stored at 4°C.

Screening for L-asparaginase production

All actinomycetes strains were evaluated for their ability to produce L-asparaginase according to the procedure of Khamna et al. (2009). Each strain of actinomycetes was inoculated on glycerol asparagine agar (Pridham and Lyons, 1961) incorporated with pH indicator; pH was adjusted to 7.0 and incubated at 30°C for seven days. Colonies with pink zones were considered as L-asparaginase-producing active strains. Two control plates were also prepared using glycerol asparagine agar; one was without dye while the other was without asparagine. The more potent strains were selected for fermentation process.

Fermentation procedure

Fermentation was carried out for the active actinomycetes strains, using 250 ml capacity Erlenmeyer flasks, containing 50 ml of glycerol asparagine medium. Each flask was inoculated by 1 ml of spore suspension (2.0×10^6 spores/ml) of three days old culture. Inoculated flasks were incubated at 30°C for five days on a rotary shaking incubator at 250 rpm. Samples were taken periodically

every day for determination of L- asparaginase activity.

Determination of L-asparaginase activity

The activity of produced L-asparaginase was assessed according to the method of Mashburn and Wriston (1963). A mixture of 0.1 ml of enzyme extract, 0.2 ml of 0.05 M Tris-HCl buffer (pH 8.6), and 1.7 ml of 0.01 M L-asparagine was incubated for 10 min at 37°C. The reaction was stopped by the addition of 0.5 ml of 1.5 M trichloroacetic acid. The ammonia released in the supernatant was determined colorimetrically at wavelength of 480 nm.

Purification of L-asparaginase with HPLC system using sephadex G-200

Ammonium sulfate was added to the crude extract from cultures grown on glycerol asparagine broth medium at 4°C to purify the L-asparaginase. The L-asparaginase activity was associated with the fraction precipitated at 70% saturation. The precipitate was collected by centrifugation at 10,000 rpm for 20 min, dissolved in 50 mM Tris-HCl buffer pH 8.6 and dialyzed against the same buffer. Using HPLC system, the concentrated enzyme solution was applied to the column of sephadex G-200 (1.5x45 cm) (Pharmacia fine co., Uppsala, Sweden) that was pre-equilibrated with 50 mM Tris-HCl buffer pH 8.6. The protein elution was done with the same buffer at a flow rate of 3 ml/30 min. It was eluted with NaCl gradient (0.1-0.5 M) and 0.1 M borate buffer at pH 7.0. The active fractions were collected, dialyzed and concentrated. The protein content was estimated by the method of Lowry et al. (1951) using bovine serum albumin as the standard.

SDS-polyacrylamide gel electrophoresis

The molecular weight of the purified L-asparaginase sample was determined by using 10% SDS-PAGE according to the method of Laemmli (1970).

Characterization of the purified L-asparaginase

The independent parameters were evaluated at a time keeping other parameters constant. The optimized parameter obtained (based on highest enzyme activity achieved) was incorporated in the next experiment while optimizing the next parameters.

Optimum pH for L- asparaginase activity

Fixed volume of 0.1 ml of the purified enzyme solution was mixed with 0.2 ml of 0.05 M buffers of different pH from 5 to 11. Potassium phosphate was used for pH range of 5 to 7, Tris-HCl was used for pH range of 8 to 9 and glycine-NaOH was used for pH range of 10 to 11, respectively. The optimum pH for L-asparaginase activity was studied by the method of Mashburn and Wriston (1963).

Optimum temperature for L-asparaginase activity and stability

Optimum temperature for the enzyme activity was determined by incubating the assay mixture for 30 min at temperatures ranging from 20 to 70°C. After the end of the incubation periods, enzyme activity was measured as previously mentioned in optimum pH for L-asparaginase activity.

To study the optimum temperature for L-asparaginase stability, only modification of incubation temperature, incubation time and

substrate of the enzyme were made. The solution mixtures of 0.1 ml of enzyme solution and 0.1 ml of 0.05 M Tris-HCl buffer pH 8 was incubated for 15, 30, 45, 60, 90, 120, 150 and 180 min at different degrees of temperature varying from 30 to 70°C in 10°C increments and was directly cooled in ice bath. Enzyme activity of each tube was measured as previously described (Aljewari et al., 2010).

Effects of metal ions and some inhibitors on L-asparaginase activity

To determine the effect of different metal ions (K^+ , Ca^{+2} , Fe^{+2} , Zn^{+2} , Cu^{+2} , Hg^{+2} and EDTA), different salt solutions ($KMnO_4$, $CaCl_2$, $FeSO_4$, $ZnCl_2$, $Cu(C_2H_3O_2)_2$, $Hg(C_2H_3O_2)_2$ and EDTA) were added to the enzyme-substrate reaction mixture to yield final concentrations of 0.25, 0.5, 1, 5, and 10 mM. The mixtures were incubated for 30 min. and L-asparaginase specific activity of each sample was then determined.

Kinetics of the purified enzyme

To measure the kinetics of L-asparaginase, Michaelis constant (K_m), and maximal velocity (V_{max}) of the purified enzyme was determined. They are one of the important parameters for the evaluation of the potential usefulness of the enzyme for anti-leukaemic therapy. They were determined using L-asparagine as substrate in the range of 0.2 to 4.0 mM. Each reported reaction velocity is the mean of at least three measurements. The apparent K_m was calculated from the double-reciprocal Lineweaver-Burk plot (Basha et al., 2009).

Determination of anti-tumor property of the enzyme

***In-vitro* assessment of anti-tumor activity**

The anti-tumor activity of the purified L-asparaginase preparations was determined *in vitro* against Ehrlich ascites carcinoma (EAC); a cell line which was kindly provided by the National Cancer Institute, Cairo, Egypt. Briefly, EAC cells were suspended in RPMI-1640 complete medium (each 100 ml contains 10% fetal bovine serum, 10 µg/ml streptomycin and 100 U/ml penicillin) and seeded in 96 flat-bottomed wells plate at a concentration of 2×10^6 /well. 200 µL of different L-asparaginase preparations (10, 20 and 50 U/ml) were seeded in triplicates and then incubated for 24 h in 5% CO_2 incubator at 37°C (El-khawaga et al., 2003). Cells viability was checked by using trypan blue staining and cytotoxicity values were calculated (Boyum, 1967).

***In-vivo* anti-tumor activity**

Female Albino mice with body weight of 20 to 22 g were injected with 2×10^5 EAC cells subcutaneously (S.C.) between thighs to induce solid tumor according to Mohamed et al. (2003). Animals were randomly divided into three groups (8 mice each). 24 h after EAC inoculation, one group of animals was intraperitoneal (i.p.) injected with 0.2 ml of 50 U/ml L- asparaginase preparation for five consecutive days. Another group of animals was intraperitoneally treated with 0.2 ml of anticancer drug thalidomide (1.25 mM/kg) for five consecutive days and served as control group. The tumor positive control group was treated with 0.2 ml of 0.9% normal saline. After three weeks from EAC implantation, animals were sacrificed and solid tumors were excised and weighed. Also, livers were removed, rinsed with 0.9% physiological saline and kept at -

20°C and used for lipid peroxidation determination and assessment of the activity of antioxidant enzymes (CAT and SOD).

Preparation of liver homogenate

A liver tissue of 0.5 g was cut into small pieces and homogenized in 5 mL of cold phosphate buffered saline, pH 7.2, by using mechanical homogenizer. Liver homogenate was centrifuged at 8000 rpm at 4°C for 5 min, and then, the supernatant was transferred into small aliquots and kept at -70°C.

Determination of antioxidant enzymes activity in liver homogenate

Hepatic CAT activity was determined according to the method described by Slaughter and O'Brien (2000). Briefly, the remainder H_2O_2 was assayed spectrophotometrically in the presence of a chromogen at 560 nm after reaction of the catalase enzyme with definite amount of H_2O_2 . Results were expressed as U/g tissue. Meanwhile, hepatic SOD activity was assayed according to the method described by Rest and Spitznagel (1977). This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye, the produced color measured at 560 nm. Results were expressed as U/g tissue.

Determination of hepatic malondialdehyde (MDA) level

Malondialdehyde (MDA) as the end product of lipid peroxidation was estimated in liver homogenate according to the procedure of Ohkawa et al. (1979). The reaction mixture contained 0.1 ml sample, 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 20% acetic acid, and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid. The pH of the mixture was adjusted to 3.5 and the volume was finally made up to 4 ml with distilled water and 5 ml of the mixture of n-butanol and pyridine (15:1, v/v) was added. The mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 min, the absorbance of the organic layer was measured at 532 nm. Results were expressed as nmol/g tissue.

Statistical analysis

The statistical significance of the experimental biochemical results was determined by the Student's t-test Murray (1982). For all analyses, $p < 0.05$ was accepted as a significant probability level.

RESULTS

Isolation of actinomycetes and L-asparaginase activity

Preliminarily screening showed that six strains of actinomycetes exhibited L-asparaginase activity. All active strains were cultivated in glycerol-asparagine broth. Production of L-asparaginase began after 72 h of cultivation (1.3 U/ml) and reached to maximum level (3.9 U/ml) after 120 h of incubation. Out of the six active strains, *S. halstedii* (data of identification not showed here) showed highest L-asparaginase activity (8.643 µmol ammonia/ml) after 120 h of incubation.

Table 1. Effect of different concentrations of ammonium sulfate on L-asparaginase activity, protein content and specific activity.

Concentration of ammonium sulfate%	Activity (U)	Protein content (mg)	Specific activity (U/mg protein)
40	9.88 ±0.35	0.62 ±0.03	15.93 ±0.02
50	19.10 ±0.26	0.76 ±0.03	25.13 ±0.35
60	22.36 ±0.33	0.82 ±0.03	27.27 ±0.24
70	40.97 ±1.08	0.93 ±0.02	44.05 ±0.16
80	37.29 ±0.6	1.45 ±0.06	25.72 ±0.09
90	33.47 ±1.01	1.87 ±0.04	17.9 ±0.4

Purification of L-asparaginase

Optimization of ammonium sulfate precipitation

The results presented in Table 1 show that as ammonium sulfate concentration increased, L-asparaginase activity and the specific activity in the precipitates increased. They reached their maximum value at 70% saturation, then, they decreased by increasing ammonium sulfate concentration above 70%. So the 70% saturation was selected for the first step in the purification process of the enzyme.

Purification of L-asparaginase with HPLC system using sephadex G-200

Purification of L-asparaginase was carried out in three steps as shown in Table 2. Purification of L-asparaginase crude extract as affected by ammonium sulfate (70%) precipitation showed that, most of the enzyme activity was preserved in the precipitate. Total protein decreased from 2.3 to 1.86 mg, and specific activity increased from 37.5 to 228.71 U/mg protein, at approximately 6.09 folds purity in the ammonium sulfate precipitation step.

Precipitated enzyme sample was further purified with sephadex G-200 using HPLC system. Figure 1 shows that active fractionations started from fractions 14 -18. HPLC system purification step resulted in 2071.3 fold increase in specific activity of L-asparaginase produced by *S. halstedii* and the purification fold was also increased to 55.2 (Table 2). All purification steps produced an increase in specific activity.

Determination of protein molecular weight of the purified enzyme

Figure 2 shows the protein profile analysed by 10% SDS-PAGE. Analysis of the gel revealed that there was no detectable contamination as it represented just one distinct band and the molecular weight of the band of 100 kDa.

Characterization of the purified L-asparaginase

Optimum pH

The results of L-asparaginase activity in different pH values showed that the enzyme has quite a wide range of activity between 5 and 10 (Figure 3). At pH below 5 and above 10, the enzyme activity was very low. At pH around 6 and 9, the activity drops to about 60% of its maximum. Also, according to Figure 3, the profile of enzyme activity showed that there was an increase in the enzyme activity as pH increased until it reaches its optimal activity at pH 8.

Optimum temperature for L-asparaginase activity and stability

Optimum temperature of L-asparaginase is given in Figure 4. It showed that the enzyme was active at a wide range of temperature condition from 20 to 70°C. The optimum temperature for L-asparaginase activity was found to be 37°C. At higher temperatures, the reaction rate declined sharply.

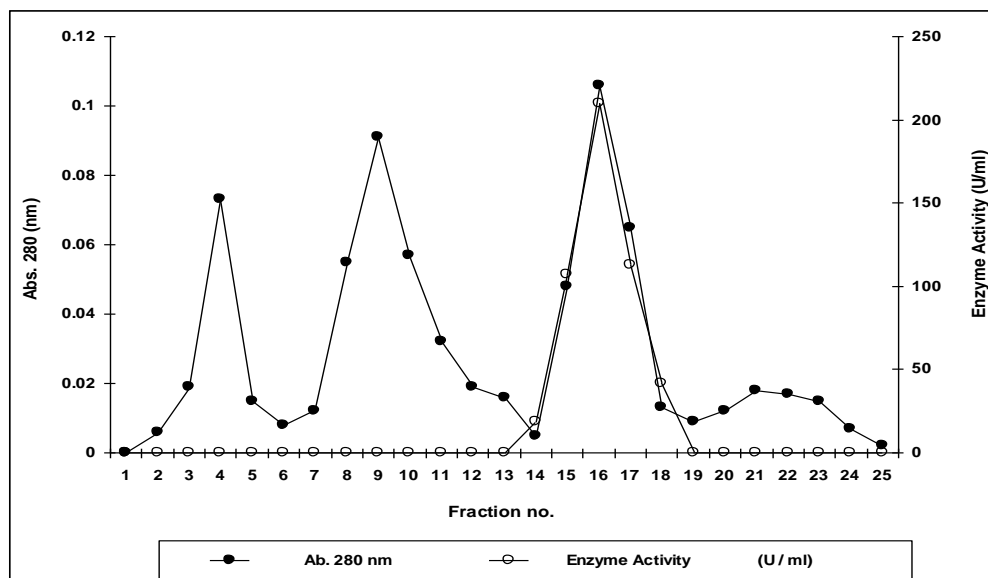
Thermal stability behavior of L-asparaginase as a function of heating time is shown in Figure 5. The data indicated that no significant enzyme activity was lost when it was pre incubated at 30 and 40°C for 180 min. At 50°C, about 80% of the enzyme activity was retained at 150 min incubation time. The enzyme lost about 50% of its residual activity when it was pre incubated at 60°C for 150 min. while at 70°C the enzyme lost about 50% at 45 min incubation.

Effects of some inhibitors and metal ions on the activity of the purified L-asparaginase enzyme

Several concentrations of EDTA (0.25-10 mM) were used to check its effect on the activity of the purified enzyme. The data of Table 3 shows that as the concentration of EDTA increases, the activity gradually decreases; at 10 mM of EDTA, the enzyme retained 52% of its activity. L-

Table 2. Purification profile of L-asparaginase from *S. halstedii*.

Purification step	Volume (ml)	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification folds	Yield (%)
Crude extract	100	86.4 ±1.41	2.3 ±0.28	37.56 ±5028	1.0 ±0	100.0 ±0
Ammonium sulfate precipitation (70%)	10	425.4 ±1.64	1.86 ±0.04	228.71 ±4.04	6.02 ±0.11	492.36 ±1.9
Sephadex G-200 filtration	3	360.4 ±1.51	0.174 ±0.004	2071.26 ±56.35	54.52 ±1.49	417.1±1.75

**Figure 1.** Elution profile of preparations obtained from *S. halstedii* by ammonium sulphate (70% saturation) using Sephadex G-200 gel filtration chromatography.

asparaginase activity was assayed in the presence of various ions and reagents (Table 3). Among the tested ions, Ca^{+2} and Fe^{+2} acted as enzyme inducer except at concentration of 10 mM of FeSO_4 , it retained 91% of its activity. However, the enzyme retained about 50% of its activity when Zn^{2+} and Cu^{2+} ions were used at 10 mM concentration as well as EDTA. Moreover, the enzyme retained about 20 and 36% of its activity when 10 mM of Hg^{++} and K^+ were used, respectively.

Kinetic parameter

The K_m and V_{max} of purified L-asparaginase from *S. halstedii* were 0.1939 mM and 1.22 mM/min, respectively (Figure 6).

In-vitro cytotoxic effect of L-asparaginase

As indicated in Table 4, there was a gradual decrease in the viability of EAC cell with increasing doses of the

purified L-asparaginase *in vitro*. As the concentration of L-asparaginase increased, the rate of cell proliferation decreased as a result of increased inhibition efficiency on cell growth.

Effect of the purified L-asparaginase on growth of solid tumor

Table 5 shows the effect of the purified L- asparaginase on the growth of solid tumor induced in female Albino mice. The results show a significant reduction in tumor weight as compared to that of tumor control group. Moreover, L-asparaginase exhibit potent inhibitory action on the tumor growth as compared to that of thalidomide anti-tumor drug which used as a reference control.

Effect of the purified L- asparaginase on the oxidative status

Table 6 shows the effect of L-asparaginase on the level

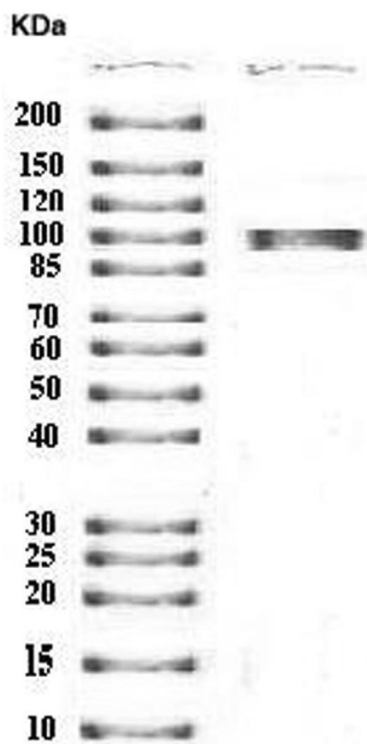


Figure 2. SDS-Polyacrylamide gel electrophoresis of the purified L-asparaginase from *S. halstedii*. 1, Protein marker of molecular weight 200 KDa (Pharmacia fine co., Uppsala, Sweden), 2. Purified protein after final step of purification.

of MDA as the end product of lipid peroxidation in tumor bearing mice. Results indicate that development of solid tumor is associated with significant elevation of MDA in liver tissue as compared to that of normal control group. Treatment with L-asparaginase resulted in a significant reduction in lipid peroxidation levels. Also, treatment of tumor bearing mice with the purified L-asparaginase leads to remarkable increases in SOD and CAT activities that accompanied reduction in tumor weight, revealing their protective mechanism in tumor prevention (Table 6).

DISCUSSION

The major bioactive compounds obtained from actinomycetes are antibiotics. However, there are varieties of compounds like enzymes that may attract research interest (Basha et al., 2009).

In the present study, *S. halstedii* was selected as potent strain for the production of L-asparaginase. L-Asparaginase formation has shown a firm link to the active cell growth (Savitri and Azmi, 2003; Narayana et al., 2008; Amena et al., 2010).

The *S. halstedii* L- asparaginase precipitated by ammonium sulfate (70%). Many authors (Amena et al., 2010; Kumar and Selvam, 2011) recorded 60% ammonium sulfate saturation and 80%, respectively. The specific activity of L-asparaginase increased from the crude extract to the final Sephadex G-200 purification step, which was approximately 55.2 folds purity. Dhevagi and Poorani (2006) found that L-asparaginase from a marine *Streptomyces* sp. PDK2 has been purified 85-fold with 2.18% recovery in the final Sephadex G-200 purification step.

Dharmaraj and Sumantha (2009), Dharmaraj et al. (2009) and Dharmaraj (2011) found that purified L-asparaginase from *S. gulbargensis*, *S. albidoflavus*, and *S. noursei* MTCC 10469 exhibited molecular weights of 85, 112, and 102 kDa, respectively while *S. halstedii* L-asparaginase contained one protein band with molecular weight of 100 kDa.

The physiological pH is one of the prerequisites for anti-tumor activity (Manna et al., 1995; Siddalingeshwara and Lingappa, 2011). Under alkaline pH condition, L-asparaginase becomes a competitive inhibitor (Stecher et al., 1999). This property of the enzyme clarified that the enzyme produced by *S. halstedii* under the present study (optimum pH 8.0) has effective carcinostatic property. The maximum activity in alkaline pH may be due to the balance between L-aspartic acid and L-aspartate. L-Aspartic acid in acid pH has a greater affinity for the active site of the enzyme. In alkaline pH, the balance is shifted toward the aspartate, which is the form with less affinity to the active site enabling, a favorable balance for the connection with the substrate L-asparagine in such alkaline pH (Stecher et al., 1999).

The property of maximum activity of the L-asparaginase at 37°C may be more suitable for complete elimination of asparagine from the body when tumor patient is treated with L-asparaginase (Siddalingeshwara and Lingappa, 2011). Previous data about *P. stutzeri* MB-405, *E. coli*, *Bacillus* sp., pathogenic *E. coli*, and *Aspergillus terreus* KLS optimum temperature for L-asparaginase maximum activity was 37°C (Manna et al., 1995; Li et al., 2007; Aljewari et al., 2010; Moorthy et al., 2010; Siddalingeshwara and Lingappa, 2011) which coincide with *S. halstedii* L-asparaginase optimum temperature for maximum activity in the present study.

The effect of some metal ions and EDTA on *S. halstedii* L-asparaginase coincided to somehow that of Basha et al. (2009) who found that marine actinomycetes isolates S3, S4 and K8 were inhibited by Cu^{2+} , Zn^{2+} and EDTA. The Inhibition of enzyme activity in the presence of Hg^{2+} might be indicative of essential vicinal sulfhydryl groups (SH-group) of the enzyme for productive catalysis (Elshafei et al., 2012). These results indicate that the activity of the enzyme may depend on the presence of sulfhydryl functional groups and the enzyme may not be a metalloenzyme (Basha et al., 2009; Warangkar and Khobragade, 2010).

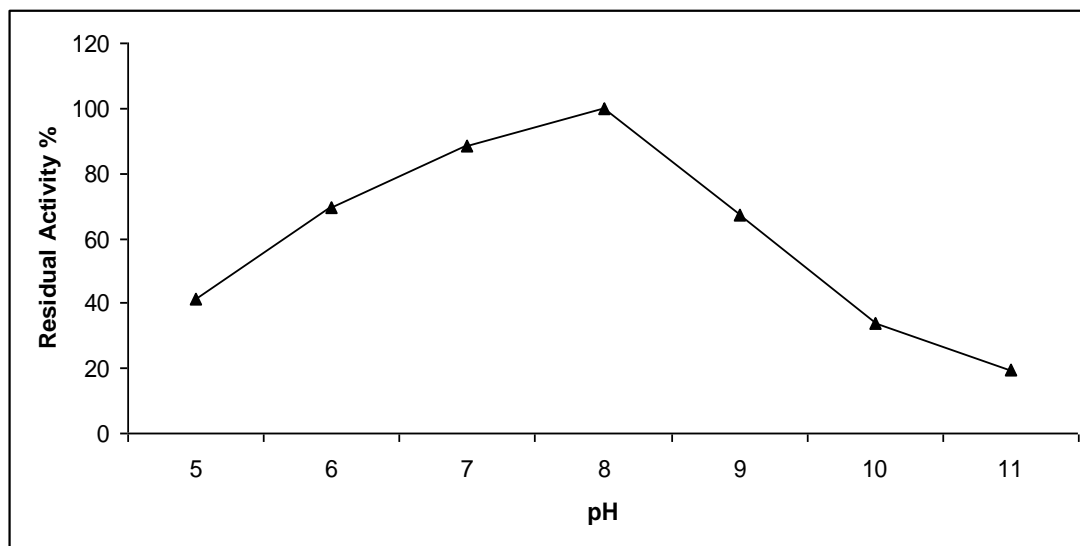


Figure 3. Effect of pH values on the purified L-asparaginase activity from *S. halstedii*.

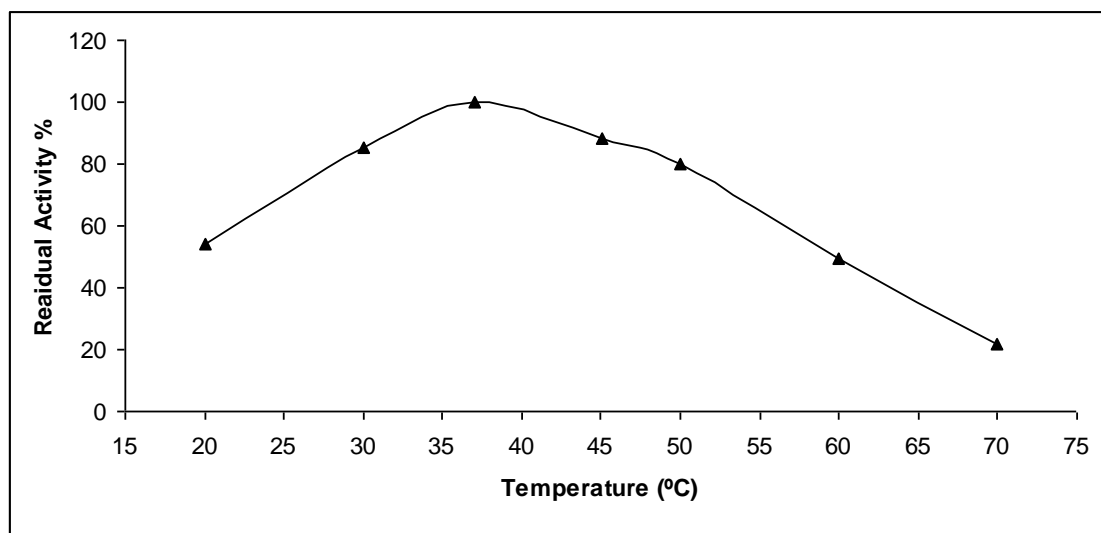


Figure 4. Optimum temperature of the purified L-asparaginase activity from *S. halstedii*.

The K_m and V_{max} of purified L-asparaginase from *S. halstedii* showed high affinity of the enzyme to the substrate (0.1939 mM and 1.22 mM/min, respectively). These kinetic parameters are comparable with those reported for many microorganisms (Basha et al., 2009; Kumar and Selvam 2011).

The purified L-asparaginase from *S. halstedii* exhibits gradual inhibition in the growth of EAC cells *in vitro* as the concentration of the enzyme increased. The inhibition of EAC cells growth coincides with that obtained by many authors (Moharam et al., 2010). Moreover, the enzyme showed a potent inhibitory action on the tumor growth as

compared to that of thalidomide anti-tumor drug which was used as a reference control.

Significant results of the antioxidants and lipid peroxidation were obtained after treatment with the purified L-asparaginase, accompanied with reduction in tumor weight, revealing their protective mechanism in tumor prevention. The status of antioxidants and lipid peroxidation were correlated with the pathophysiology of the cancer (Bandeubuche and Melinkeri, 2011). This means that, in tumor control, the increased serum MDA levels indicate oxidative stress which may cause DNA damage which is one of the causative factors for cancer

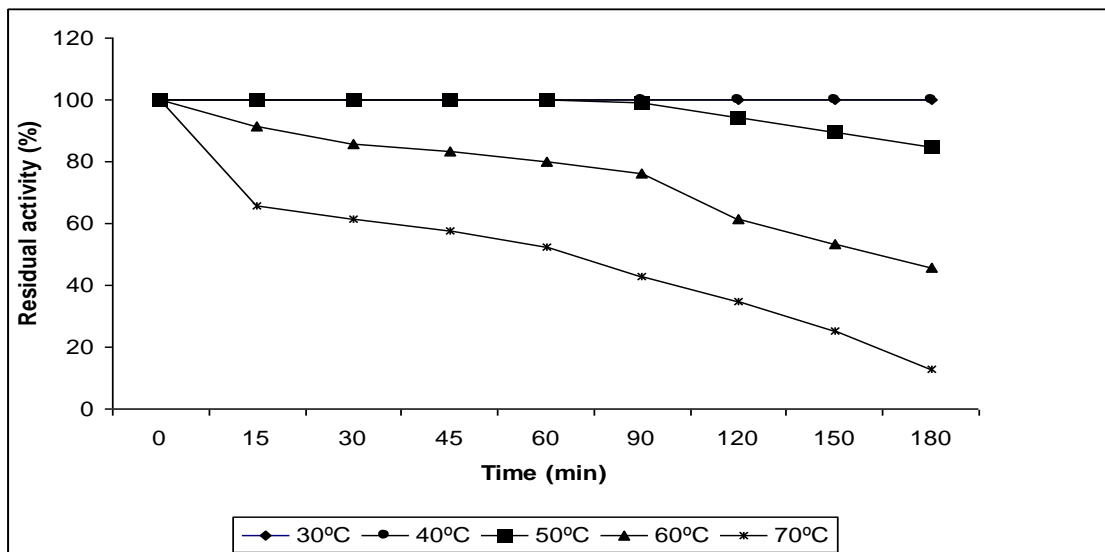


Figure 5. Thermal stability of the purified L- asparaginase from *S. halstedii*.

Table 3. Effect of different concentrations of some metal ions and EDTA on the activity of the purified L-asparaginase from *S. halstedii*.

Concentration (mM)	L-Asparaginase residual activity (%)						
	KMnO ₄	CaCl ₂	FeSO ₄	ZnCl ₂	Cu(C ₂ H ₃ O ₂) ₂ H ₂ O	Hg(C ₂ H ₃ O ₂) ₂	EDTA
0.0	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0	100 ±0.0
0.25	92.4 ±0.61	100.0 ±0.0	100.0 ±0.0	98.5 ±0.16	96.8 ±0.22	96.4 ±0.26	64.1 ±0.21
0.5	81.9 ±0.15	100.0 ±0.0	100.0 ±0.0	87.6 ±0.31	87.2 ±0.35	52.9 ±0.27	61.9 ±0.4
1.0	65.6 ±0.17	100.0 ±0.0	100.0 ±0.0	79.5 ±0.71	72.9 ±0.58	41.3 ±0.14	58.7 ±0.48
5.0	48.0 ±0.11	100.0 ±0.0	100.0 ±0.0	68.2 ±0.21	66.1 ±0.28	33.5 ±0.28	54.7 ±0.17
10.0	36.4 ±0.45	100.0 ±0.0	91.3 ±0.47	56.6 ±0.38	55.0 ±0.11	20.2 ±0.37	52.0 ±0.66

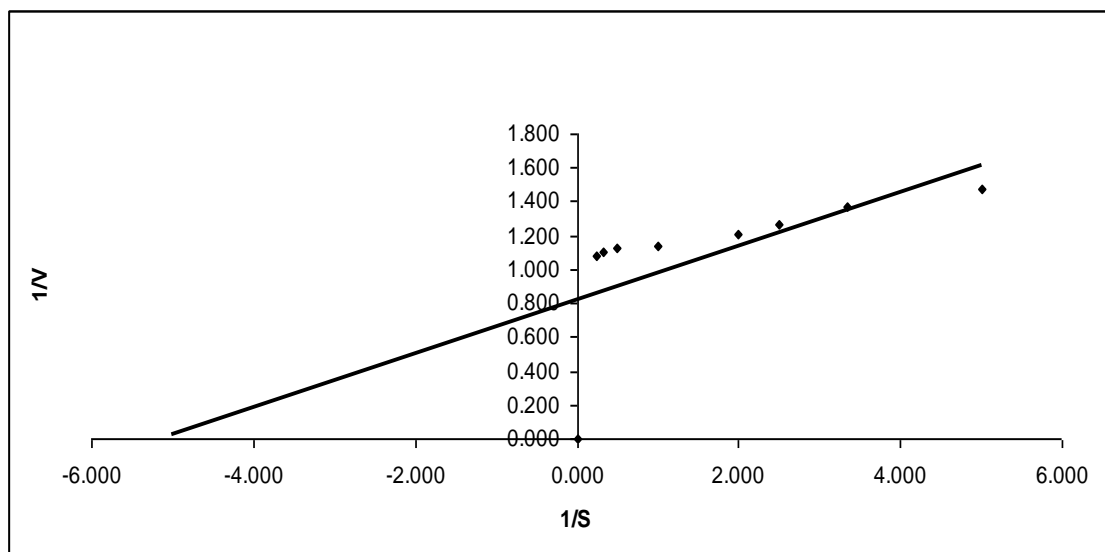


Figure 6. Lineweaver-Burk plot of L-asparaginase from *S. halstedii*. V is the reaction rate and S is the concentration of the substrate.

Table 4. The cytotoxic effect of the purified L-asparaginase on EAC cells *in-Vitro*.

Purified L-asparaginase (U/ml)	Cytotoxicity %
10	27.4 ± 6.9
20	50.4 ± 7.3
50	83.4 ± 6.6

Table 5. Effect of the purified L-asparaginase on the growth of solid tumor.

L-asparaginase	Tumor weight (g)	Inhibition%
<i>S. halstedii</i>	0.57 ± 0.13	74.10
Thalidomide	1.31 ± 0.42	40.45
Control	2.20 ± 0.31	-

Table 6. Effect of L-asparaginase and thalidomide on hepatic oxidative status.

Treatment	MDA (nmol /g tissue)	CAT (U/g tissue)	SOD (U/g tissue)
Normal control	69.1 ± 11.0	7.7 ± 1.5	32.6 ± 2.5
Tumor control	197.5 ± 10.8*	3.1 ± 0.5*	19.8 ± 7.0*
<i>L-asparaginase</i>	54.8 ± 4.6\$	8.1 ± 0.3\$	65.0 ± 6.1\$
Thalidomide	188.5 ± 19.0ns	3.5 ± 0.7ns	16.7 ± 3.1ns

*Significant when compared with normal control; \$, Significant when compared with tumor control; ns, Non-significant when compared with tumor control.

while low levels of SOD and CAT could be due to the increased utilization of these antioxidants in scavenging the lipid peroxides production which overrides the antioxidant defense leading to increased MDA in serum (Bandeubuche and Melinkeri 2011).

In conclusion, this study demonstrates the potential anti-tumor activity of L-asparaginase isolated and purified from strain *S. halstedii* from Egyptian soil. This enzyme exhibited a cytotoxic effect against cancer cell line *in vitro* and in addition, it reduced the growth of solid tumor induced in albino mice. The reduction of tumor size may be attributed to the elevation of CAT and SOD activities as well as the diminishing of MDA.

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