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Fusarium sp. L-Asparaginases: purification, characterization and potential assessment as anti-leukemic chemotherapeutic agent

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Research Article

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Abstract

Asparaginases play an important role in the treatment of leukemia. It is part of chemotherapy in the treatment of leukemia in the last three decades. L-Asparaginase isolated from *Fusarium sp*. isolated from soil and purified using ammonium sulfate precipitation and Sephadex G 100. Characterization of the crude enzyme revealed it is a metalloprotease inhibited by EDTA. $Hg2^{+}$, $Cd2^{+}$, and $Pb2^{+}$ also inhibited the enzyme. $Mg2^{+}$, $Zn2^{+}$ and $Ca2^{+}$ activated L. asparaginase. Further, the kinetic studies of purified enzyme were carried out. V_{max} and K_m and were 0.031 M and 454 U/mL, respectively. The optimum temperature was 30°C, optimum pH was 7. Concerning substrate specificity; gelatin and casein in addition to L-asparagine were tested. The enzyme was found to be non-specific, could hydrolyze all tested substrates at different rates. Maximum enzyme activity was recorded in the case of L-asparagine, followed by Casein and gelatin, respectively. The molecular weight of L-Asparaginase was 22.5 kDa. The Anti-leukemic cytotoxicity assay of the enzyme against RAW2674 leukemic cell lines by MTT viability test was estimated. The enzyme exhibited anti-leukemic activity with IC_{50} of 70 Uml^{-1} and relative viability 70 % of control. The current work presents additional information regarding the purification and characterization of the enzyme produced by *Fusarium sp.* and its evaluation as a potential anti-leukemic chemotherapeutic agent.

Introduction

L-Asparaginase is a metabolism-targeted anti-neoplastic agent help in the treatment of leukemia. This is due to the enzymatic breakdown of glutamine and asparagine, which is changed in the blood to glutamic acid and aspartic acid, respectively (Covini et al. 2012; Lopes et al. 2017). L-Asparaginase converts L-asparagine to aspartic acid and ammonia. It is used in chemotherapy to treat leukemia and lymphomas (El-Nagga et al., 2014). L-Asparaginase is found in animals, plants, microorganisms. It is used as a chemotherapeutic agent to treat lymphoma and lymphoproliferative disorders (Cachumba et al., 2016).

L-Asparaginase is an amid hydrolase enzyme that deamidates L-asparagine to L-aspartic acid and ammonia and L-glutamine to L-glutamic acid and ammonia. The enzyme can hydrolyze asparagine, causing the death of tumor cells, which lack asparagine synthetases. (Costa et al., 2016). It hydrolyses L-asparagine to ammonia and aspartic acid. The mechanism of the enzyme is due to the hydrolysis of L-asparagine, where tumor cells cannot form asparagine and are killed. This leads to susceptibility to the cytotoxicity of L-Asparaginase due to inhibition in protein synthesis (El-Nagga et al., 2014).

Many factors affect the L-asparaginase therapeutic properties, including L-asparaginase sources, upstream and downstream bioprocessing, tumor cell resistance, anti-asparaginase antibiotics, and hydrolysis rate. Km (Michaelis constant), clearance of the serum, pharmacological aspects, and hepatic asparagine de novo biosynthesis (Lopes et al. 2017). Enzyme biological production involves multiple catalytic reactions, which make it a sophisticated process. Several parameters have to be taken into account for selecting a commercially viable source of L-asparaginase, such as environmental conditions, biochemical parameters, and genetic influence(Cachumba et al., 2016).

Since it was first recorded in *E. coli*, microorganisms have become the most abundant source of L-Asparaginase (Mashburn and Wriston 1964). Microbes generate distinct asparaginase types that vary in their cellular locations and features, namely (periplasmic, extracellular, intracellular, and glutaminase asparaginase) that have a role to play in fundamental metabolism (Orabi et al. 2019b). including bacteria and fungi: *Erwinia caratovora* (Pieters et al. 2011), *Escherichia coli* (Duval et al. 2002) *Aspergillus niger* (Luhana et al. 2013),*Aspergillus nidulans, Aspergillus tamari* and *Aspergillus terreus*, (Sarquis et al. 2004a), *Penicillium* sp. (M.Sc et al. 2012) H*elminthosporium, Paecilomyces, Alternaria* and *Cladobotrytis* (Gupta et al. 2009).

L-asparaginase from the terrestrial and marine environments has been reported from both gram-positive and gram-negative bacteria. Gram-positive bacteria were less investigated than Gram-negative bacteria(Ameen et al., 2021). L Asparaginase can be classified into two main types, type I and type II asparaginase, in most gram-negative bacteria. Type I L-asparaginase is quantitatively expressed and has both L-glutamine and L-asparagine amino acid enzymatic activity. In comparison, L-asparaginase Type II has high specific L-asparagine activity and is only induced in an anaerobic state(Batool et al., 2016; Orabi et al., 2019a).

Apart from its medical usage, L-Asparaginase were also categorized as inhibitors of acrylamide formation in heated foods (Kornbrust et al. 2009); the International Agency for Research on Cancer) declared acrylamide as a potent neurotoxin and a carcinogen Implying that L-Asparaginases have future potential in the food industries (Kornbrust et al. 2009).

This enzyme's importance can also be seen from its high global demand of $380 million in 2017, which is expected to increase by $420 million by2025 (Alam et al., 2019). The present work's main objective was to assay L-Asparaginases extracted from soil Fusarium species and optimize the growth parameters to achieve maximum enzyme production, purification, characterization of the enzyme, and assess its anti-leukemic bioactivity.

Material And Methods

Fungal Isolation

Ten soil samples were collected from the soil's garden, college of Applied Medical Science, Hafr Al Batin University, Hafr Al-Batin, Saudi Arabia. One gram of each soil sample was added into 9 ml of sterile distilled water, serially diluted (10^{-2} and 10^{-3}), and 0.2 mL was plated into Potato Dextrose Agar (PDA) and incubated at 28°C for 96h (Taubeneck 2007).

Identification of fungi

Fungal colonies were purified and identified (Taubeneck 2007). Eventually, fungal species were transferred to PDA slants, incubated at 28°C for 4 days, and stored at 4°C, until use.

Culture media and growth conditions

The culture medium used for the study was a modified Czapek- Dox medium containing g/l of, Glucose, 2.0; L-asparagine, 10.0; KH_2PO_4, 1.52; KCl, 0.52; $MgSO_4$. $7H_2O$, 0.52; $CuNO_3.3H_2O$, trace; $ZnSO_4.7H_20$, trace; $FeSO_4$. $7H_20$, trace; Agar, 18.0, initial pH 5.6 supplemented with either bromo cresol purple or phenol red dyes both at 0.009% (Saxena and Sinha, 1981). The cultures were incubated at 28 °C under shaken conditions (120 rpm) for 4 days.

L-Asparaginase induction

L-Asparaginase induction was performed in 250 mL flasks with 50 mL medium. Inoculate the medium with *Fusarium* sp. Incubate flasks in shaking incubator (150 rpm) for 48 h at 37°C Whatman No. 1 harvested cultures. Nesslerization was used to assay L-Asparaginase activity(IMADA et al., 2009).

L- Asparaginase quantitative assay

The enzyme (0.5 mL) was added in reaction mixture with 0.04 M L-asparagine (0.5 mL), 0.5 M phosphate-buffer (0.5 mL), and distilled water (up to 2.0 mL). The mixture was incubated for 30 min at 37°C. The reaction was stopped by adding 1*.5* M-trichloroacetic acid (0.5 mL). 0.2 ml of Nessler's reagent and 0.1 ml of the reaction mixtures were added to 3.7 ml of distilled water. After 20 min at 20˚C, the absorbance was measured at 450 nm was measured. Ammonia released was determined. One unit of L-Asparaginase was defined as the amount of enzyme which catalyzes the production of 1 mmol of ammonia per min under the standard conditions of the assay (IMADA et al. 2009)

Enzyme purification

Two purification steps were carried out to purify L-Asparaginase, $(NH_4)_2$ SO_4 precipitation up to 80 %, gel filtration through three Sephadex columns (G 100) with elution by 0.01 M phosphate buffer pH 8.

Determination of protein

The protein was measured using Lowry *et al.* (1951). A sample of 0.5 mL was mixed with 1 mL freshly mixed 1:1 ratio solution of 2% sodium hydroxide and 0.5 % copper sulphate in 1 % sodium tartrate. The mixture was left for 10 minutes before the addition of 0.1 mL folin phenol reagent and made up to a volume. The optical density of the mixture was measured after 30 minutes at 700 nm.

Molecular mass determination

Molecular mass was determined using SDS-PAGE with coomassie brilliant blue staining method (LAEMMLI 1970).

Characterization of enzyme

Effect of temperature

Effect of temperature on L-Asparaginase production was assayed at different temperatures (10-60ºC) in 50 mM phosphate buffer (pH 7.0). The flasks were incubated for 4 days, and the supernatant was used as a crude enzyme to calculate the L-Asparaginase activity.

Thermal Stability

L-Asparaginase was incubated at 10-70˚C for an hour, and then an assay was carried out to determine the remained activity.

Effect of pH

The enzyme activity at 37°C was assayed at pH values 3- 10, using different buffers (50 mM acetate pH 3.0-5.0, 50 mM phosphate pH 6.0-8.0, and 50 mM carbonate buffer pH 9.0-10. Incubation time 4 days.

Effect of metal ions

L-Asparaginase was pre-incubated with metal ions (1 mM), including Zn $SO4_2$, Mg Cl_2, Hg Cl_2, Cd Cl_2, Pb acetate, and Ca Cl_2 at 40ºC for 20 min. After incubation, the residual activity was measured (Wang et al., 2012).

Substrate concentration

Different L-asparagine concentrations (0-0.1M) were used. The reaction mixture was incubated at optimum temperature for the enzyme, and then the enzyme activity was measured.

Determination of Km and Vmax

By using different concentrations (0- 0.12 M) of L-asparagine. The reaction mixture was incubated at optimum temperature for 1 h, and then the enzyme activity was measured using the standard assay procedure.

Substrate specificity

Enzyme activity was determined using various substrates: gelatin, casein in addition to L-asparagine. The incubation temperature was 37°C for 60 min.

Cytotoxicity assay of L-Asparaginase against RAW2674 leukemic cell line

RAW2674 leukemic (0.5×10^5 cells/ well) in media free of serum were added to 96-well microplate flat bottom, and 20 µl of different enzyme concentrations were added incubated at 37 ºC for 24 h. Then media were removed, and 3-4, 5-dimethylthiazole -2-yl-2, 5-diphenyltetrazolium bromide (MTT 40 µl) were added and incubated for 4 h. 180 µl of acidified isopropanol/well was added to MTT crystals, solubilize it, and the plate was shacked at room temperature.

Absorbance was determined at 570 nm using 96 wells microplate ELISA reader. Triplicate repeats were performed for each enzyme concentration, and the average was calculated. Relative viability: - Absorbance of treated cells / Absorbance of control untreated cells x100.

Results

Fusarium L-asparaginase two-step purification

Various methods are used for producing and optimizing L Asparaginase from various microorganisms. Conditions of reaction for enzyme production vary from one organism to another and maybe produced in or after induction. Two purification steps were carried out to purify L-Asparaginase, $(NH_4)_2 SO_4$ precipitation up to 80 %, gel filtration through three Sephadex columns (G 100) with elution by 0.01 M phosphate buffer pH 8.

In the crude enzyme activity, the activity was 340 U/ml, protein concentration was 100 mg/ml, and specific activity was 3.4U/mg. The recovery was 100%. In case of $(NH_4)_2SO_4$, the activity was 177 U/ml, protein concentration was 46 mg/ml, and specific activity was 3.84U/mg. The recovery was 52.06 %, with a purification fold of 1.13of the crude enzyme. In Sephadex G100; the activity was 145 U/ml, protein concentration was 6 mg/ml, and specific activity was 24.2 U/mg. The recovery was 42.65 %, with a purification fold of 6.30 of the crude enzyme. The two purification steps finally resulted in the appearance of one peak of activity (Table 1).

Table 1

Fusarium sp. L-Asparaginase Purification steps

Purification procedure	L-Asparaginase activity (U/ml)	Protein (mg/ml)	Specific activity (U/mg)	Purification fold crude	Recovery (%)
Crude	340	100	3.40	-	100
$(NH_4)_2SO_4$	177	46	3.84	1.13	52.06
Sephadex G 100	145	6	24.2	6.30	42.65

Molecular mass Determination of l-asparaginase

Molecular weight determination using SDS-PAGE revealed the presence of a single protein band. This indicated the complete homogeneity of the enzyme. The molecular mass was 22.5 kDa.

Characterization of the L-Asparaginase

Effect of temperature

The biochemical properties of L-Asparaginases from different organisms differ. Commonly, the optimal temperature is between 30°C and 40°C for L-asparaginase activity. From the graph, it is clear that as the temperature increases, the enzyme activity increases. The optimum temperature of L-Asparaginase was 30°C. Higher temperatures caused a decrease in enzyme activity, where the activity was lost entirely at a temperature from 50- 60°C(Fig.2).

Thermal stability

From the data in Table 2, it is clear that the enzyme activity at temperature 10°C was 180 U/mL while the residual activity recorded 81.8%. Temperature 20°C recorded enzyme activity 220 U/mL representing 100 % residual activity. An increase in temperature was accompanied by a decrease in both enzymatic activity and residual activity as well.

Table 2

Thermal stability of L-Asparaginase purified from *Fusarium* sp.

Temperature (C)	Activity of L-Asparaginase (U/mL)	Residual Activity (%)
10	180	81.8
20	220	100
30	150	68.2
37	130	59.1
40	120	54.5
50	70	31.8
60	30	13.6
70	0	0

Substrate concentration

The data in Table 3 shows that as the substrate concentration increases, the enzymatic activity increases to reach maximum (300 U/mL) at 0.06 and 0.08 M substrate concentration. Higher concentration (0.1 M) caused a decrease in enzyme activity.

TABLE 3

Effect of different L-asparagine concentrations on enzyme activity of *Fusarium* sp.

Concentration of L-asparagine (M)	Activity of L- asparaginase (U /mL)	percent of max activity
0.00	000	00.0
0.02	180	60.0
0.04	260	86.6
0.06	300	100
0.08	300	100
0.10	200	66.6

PH

Almost all the isolated and purified L-Asparaginases from microbial sources operate in a basic medium with an 8.0 to 10 PH. It is clear that as pH increases, the enzyme activity increases up to pH, where the

optimum pH of L- asparaginase extracted from *Fusarium* sp. was detected. An increase in pH value caused a dramatic decrease in enzyme activity(Fig. 3)

Metal ions

Different ions influence L-asparaginase activity in various ways. L-Asparaginase extracted from *Fusarium* sp. was completely inhibited by EDTA, Hg^{2+}, Cd^{2+}, and Pb^{2+}. Zn^{2+}, Mg^{2+}, and Ca^{2+} activated the enzyme. Maximum activation was detected in the case of Mg^{2+}, followed by Zn^{2+} and Ca^{2+} (Fig. 4).

Substrate specificity

With respect to substrate specificity, the experiment was carried out using gelatin and casein in addition to L-asparagine. The enzyme was found to be non-specific, could hydrolyze all tested substrates at different rates. Maximum enzyme activity was recorded in the case of L-asparagine, followed by Casein and gelatin, respectively.

K_m and V_{max} of L-asparginase

Knowledge of the kinetic parameters of the enzyme is necessary to understand the rate and specificity of most biological processes. Michaelis constant calculates the affinity of the substrate hydrolysis to the enzyme (Km). Whereas Vmax is the limit at which a substrate concentration of an enzyme is completely saturated. A-line weaver bulk analysis showed a k_m, and V_{max} for the enzyme was 0.031 M and 454 U/mL(Fig.6).

Cytotoxicity

Toxicity is associated with L Glutaminase activity in most L-asparaginases, resulting in the prolonged consumption of L-glutamine by the normal liver and pancreatic cells, resulting in the rise of liver enzymes and pancreatitis. The antileukemic activity of L-Asparaginase was assayed using RAW 2647 leukemia cell line using MTT viability assay. The enzyme recorded significant antileukemic activity against the used cell line. The activity was concentration-dependent. The IC_{50} was found to be 70 U/ml.

Discussion

Purification steps resulted in the appearance of one peak of activity. Molecular mass determination using SDS-PAGE revealed the presence of one protein band, indicated total homogeneity of L-Asparaginase. The molecular mass of the enzyme was 22.5 KDa (Fig. 1)

In this respect, different molecular masses of L-Asparaginase were recorded in *E. coli* (22 kDa)(Narta et al. 2007), *Thermus thermophilus* (33 kDa) (Pritsa and Kyriakidis 2001), *Helicobacter pylori* (37 kDa) (Cappelletti et al. 2008) *Withania somnifera* (36 kDa) (Oza et al. 2010), *Erwinia Carnivora* (40 kDa) (Koerholz et al. 2009), and *Saccharomyces cerevisiae* (85 kDa) (Amena et al. 2010). While The highest for *Pseudomonas otitidis* with a 205 ± 3 kDa molecular weight(Husain et al. 2015).

Complete Characterization of the L-Asparaginase was carried out. The enzyme was metalloprotease wholly inhibited by EDTA, Hg^{2+}, Cd^{2+}, and Pb^{2+}. Zn^{2+}, Mg^{2+}, and Ca^{2+} activated the enzyme. The optimum temperature was 30°C, optimum pH was 7 (Fig. 2). different ions have different impacts on L-Asparaginase activity. *Bacillus subtilis* B11-06 L-Asparaginase activity was strongly inhibited by $Fe3^{+}$. The presence of $Mn2^{+}$, $Zn2^{+}$, $Ca2^{+}$, and

$Mg2^{+}$ declined *rhizobium etli* L-Asparaginase activity (Moreno-Enriquez et al. 2012). In the presence of $Cu2^{+}$, $Cd2^{+}$, and $Hg2^{+}$ complete inhibition of l-Asparaginase activity, *P. carotovorum* MTCC 1428 was found(Kumar et al., 2011). The addition of EDTA as a metal chelating agent did not influence the activity of the enzyme. They proved that the enzyme was not a metalloprotein(Singh and Srivastav 2013; Chohan and Rashid 2013).

L-Asparaginase obtained from most microbial species has an optimum temperature range of 37–40°C; however, *Thermococcus kodaka* TK1656 and *Thermococcus*

gammatolerans EJ3 at 85°C generate thermostable L-Asparaginase(Zuo et al. 2014). Maximum L-Asparaginase activity was at 35°C at pH 5, KH_2PO_4 (0.25%), Mg^{2+} (0.002%) and sucrose (1%)(Ruma et al. 2017) .(Sarquis et al. 2004b) reported that 30°C was the optimum temperature for L-Asparaginase induction for *Aspergillus terreus and A. tamarii*. While (Siddalingeshwara and Lingappa 2011) observed a temperature of 37°C was optimum for Aspergillus species' asparaginase activity. (S More 2013) Stated the maximum activity of L-Asparaginase of *Mucor hiemalis* was 30°C.

the optimum temperature was 30°C for *Penicillium* sp. (Shrivastava et al., 2012). (Chandrasekhar 2012) showed the maximum activity of L-Asparaginase of *Aspergillus* species was pH 7.

A research study found the optimum pH for L-asparaginase production by *Fusarium* species was 6.2 (Thirunavukkarasu et al., 2011). Also, the optimum pH for L-Asparaginase production in *Penicillium* species was 6.0 (Lincoln et al., 2019). While the optimum pH for L-Asparaginase production by *F. oxysporum* was 5.0 (Yadav and Sarkar 2014). Interestingly, the optimum pH for asparaginase production by *Aspergillus Terreus* was 8. (Farag et al. 2015)

Almost all the isolated and purified L-Asparaginases from microbial sources operate in a basic medium with an 8.0 to 10 pH. However, the extracted enzyme from *E.coli* was found to work maxim at PH 6, and *Bacillus licheniformis* produces L-Asparaginase efficiently in slightly acidic to strongly basic medium conditions 6.0–10 pH(Borah et al. 2012). The kinetic parameters of the enzyme must be defined to understand the rates and specificity of most biological processes, aside from gaining knowledge concerning different properties of the enzyme during a biochemical interaction.

The kinetic parameters of the enzyme must be carefully investigated for different industrial approaches. Such parameters rely on different factors, including PH, microbial origin, temperature, and substrate concentration (Miskovic et al., 2019). Substrate specificity was carried out using gelatin and casein in

addition to L-asparagine. The enzyme was non-specific could hydrolyze all tested substrates. A-line weaver bulk analysis showed a k_m, and v_{max} for the enzyme was 0.031 M and 454 U/mL (Fig. 6).

L-Asparaginase isolated of *Pichia polymorpha* had a Km value of 13.7 M and optimum pH of 6.7 (Foda et al., 1980). On the other hand, L-Asparaginase produced by *Candid utilis* had Km 7.7 M (Sakamoto et al. 1977). L-Asparaginase from *Streptomyces brollosae* showed maximum activity at pH 8.5. The optimum temperature of 37°C., Km value of 0.021 M.; Vmax of 152.6 UmL-1min-^{1} (El-Naggar et al. 2018).

The antileukemic cytotoxicity assay of L-Asparaginase was carried out using RAW 2647 leukemia cell line using MTT viability assay. The enzyme showed significant antileukemic activity. The antileukemic activity was concentration-dependent. The IC_{50} of the enzyme was 70 U/ml (Fig. 7).

Vast amounts of L-Asparaginase are needed by lymphatic cells to keep their malignant growth as it lacks L-asparagine synthetase. Healthy cells can synthesize L-asparagine so not affected (Narta et al. 2007). L-Asparaginase arrest cell cycle in G1 phase in the leukemia murine cell line (Ueno et al. 1997). The cells of CCRF-CEM showed remarkable apoptotic properties after 24 h exposure, including cell shrinking, chromatin condensation, and loss of typical nuclear architecture (Prakasham et al. 2010).

For successful clinical studies, L-glutaminase free-L-Asparaginase is highly desirable (Distasio et al., 1982). L-Asparaginase from *Pectobacterium carotovarum* is known to be an excellent anticancer agent (Kumar et al. 2009). L-glutaminase in L-Asparaginase is the leading cause of various side effects of this antileukemic drug (Manna et al. 1995). L-glutamate and ammonia generate from L-glutamine hydrolysis may also contribute to the toxicity of L-Asparaginase in leukemic patients (Richards and Kilberg 2006).

L-Asparaginase importance not only implemented in healthcare and pharmaceuticals but also food industries. To tackle the concerns caused by the unwanted acrylamide production, a carcinogen in particular foodstuffs, such as potato chips and all rich carbohydrates food(Xu et al. 2016). The exposure of millions of people to these products on a daily basis involves severe risks and critical concerns (Adebo et al., 2017).

Studies confirmed the acrylamide formation caused by a Millard reaction between l-asparagine and carbonyl compounds that take place at high temperatures in food sources rich in carbohydrates. L-Asparaginase breaks down asparagine into aspartic acid, and ammonia successfully lowers acrylamide levels in potatoes and various bakery products(Pedreschi et al. 2008).

The processing of such products with pure asparaginase like gingerbread has reduced the amount of free asparagine by nearly 70–75% and the level of acrylamide in baked products by 50–55%(Adebo et al. 2017). Despite the potential anti-leukemic activity of L-Asparaginase, it causes certain side effects. Where the administration of L-Asparaginase by leukemic patients causes normal cell lethalness(Narta et al. 2007). The enzyme causes a wide range of symptoms, including edema, pancreatic dysfunction, hepatic disorders, neurological convulsions, fever, and skin rashes. Moola recorded some hypersensitivity

interactions, mild allergic actions, and anaphylactic shock caused by the administration of asparaginase-based drugs.

L-Asparaginase-induced neurotoxicity appears to be more likely in adolescents, resulting in depression, lassitude, brain fog, blurred vision, and agitation(Battistel et al. 2020; Wardani et al. 2020). It is essential to mention here that the glutaminase effects are supposed to lead to the toxicity of L-Asparaginases. L-Asparaginase is highly immunogenic with a short half-life. Furthermore, tumor cells were reported to intracellularly produce l-asparagine, which resists the effects of L-Asparaginase(Battistel et al. 2020). The enzyme is also sensitive to thermal and proteolytic digestion and is sometimes denatured by organic solvent exposure. Recombinant molecular cloning and enzyme genetic engineering perpetual improvements could help us overcome such limitations(Zhang et al. 2004; Batool et al. 2016).

Conclusion

Fusarium sp. L- asparaginase can be used as a substitute for bacterial L-Asparaginase. It showed potent antileukemic activity and had a low IC_{50} value. L-Asparaginase retrieved from various sources is very crucial due to its anti-cancer and tumor cells therapeutic effects. Besides pharmaceutical usage, in the food industries, it is used to efficiently delay the production of acrylamide in foodstuffs rich in carbohydrates. L-Asparaginase recovered from diverse microbial sources marginally vary in biochemical and physical features with distinct kinetic parameters.

The alterations in the enzyme's physiochemical and kinetic characteristics have afforded us the possibility to search and characterize this enzyme according to the requirements. Some sources provide the efficient working of L-Asparaginase at 37°C, the human body's normal temperature. Therefore, L-Asparaginase from such sources could be purified and used in healthcare industries.

While the highly thermostable enzyme version can be used in industrial applications that usually demand high temperatures, although L-Asparaginase has become widely used as an anti-cancer medicine and is used in the food industry, its stability and activity are affected by certain limitations. Therefore, it requires sufficient improvements in the enzyme engineering techniques to generate highly versatile versions of the enzyme at the molecular level.

The outlook of L-Asparaginase studies is designed to enhance L-Asparaginase activity and eliminate glutamine and immunosuppressive complications. For a wide range of industrial applications, highly thermostable enzyme versions are currently being genetically engineered.

Furthermore, L-Asparaginase outputs are usually insufficient low as this enzyme's gene expression rate is shallow. In addition, L-Asparaginase is used in the food industry as an aid for the eradication of carcinogenic acrylamide compounds. It is, therefore, our time to explore and optimize production and purification parameters from new enzyme sources so that more potential and specific L-Asparaginase enzyme production can be developed.

By better understanding of recombinant technologies such as molecular cloning and genetic engineering, novel enzymes with far less immunogenic consequences, unique functionalities, and better half-life could be designed. Finally, using recent recombinant technology could help increase the net output of this powerful therapeutic enzyme.

Declarations

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Compliance with ethical standards

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Figures

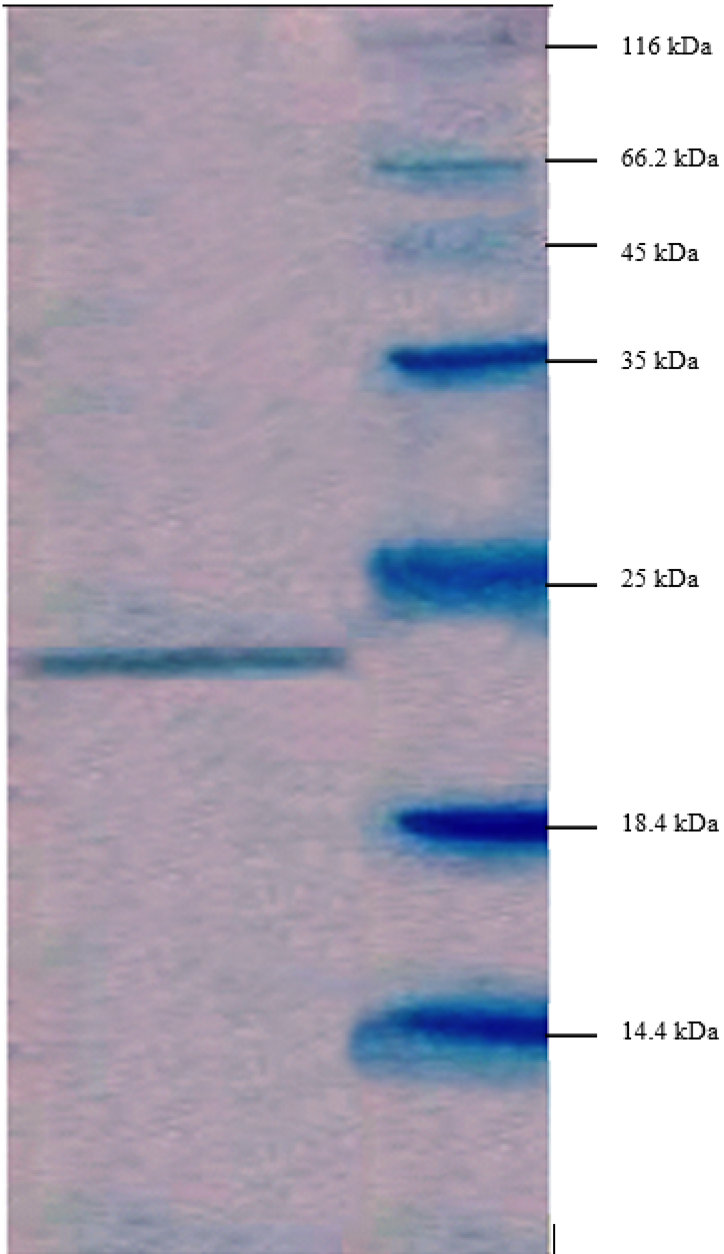

Figure 1

Molecular mass of the purified L- asparaginase

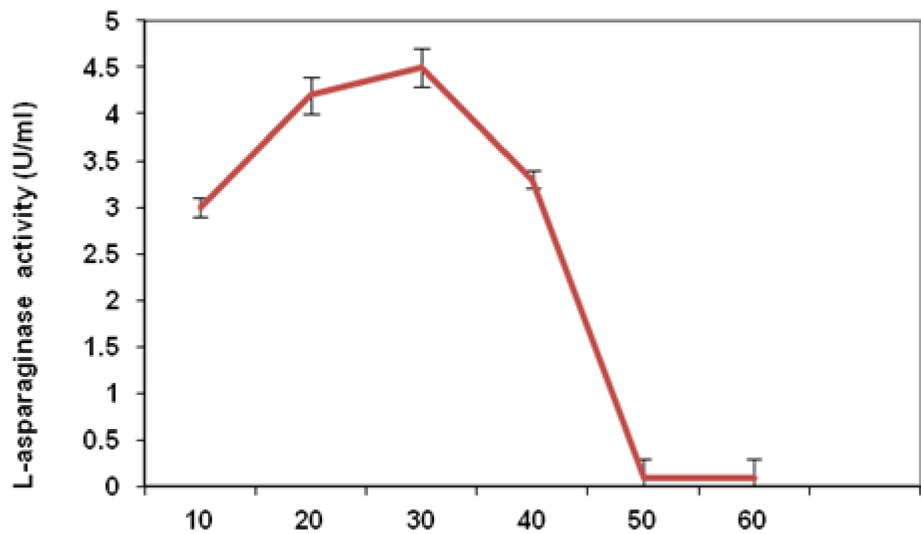

Figure 2

Effect of temperature on L-Asparaginase activity

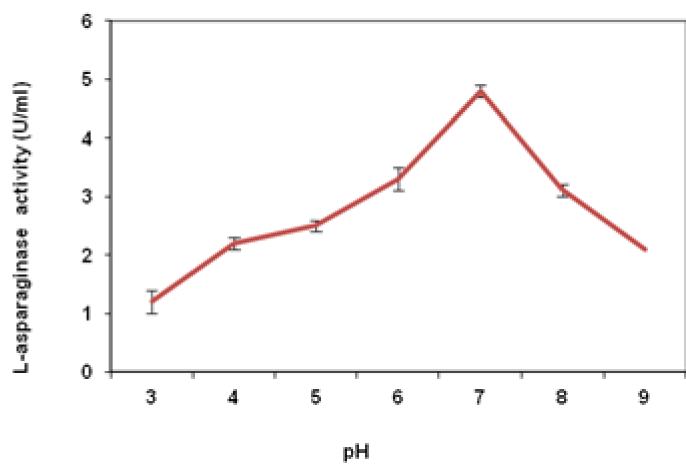

Figure 3

Effect of PH on L-Asparaginase activity

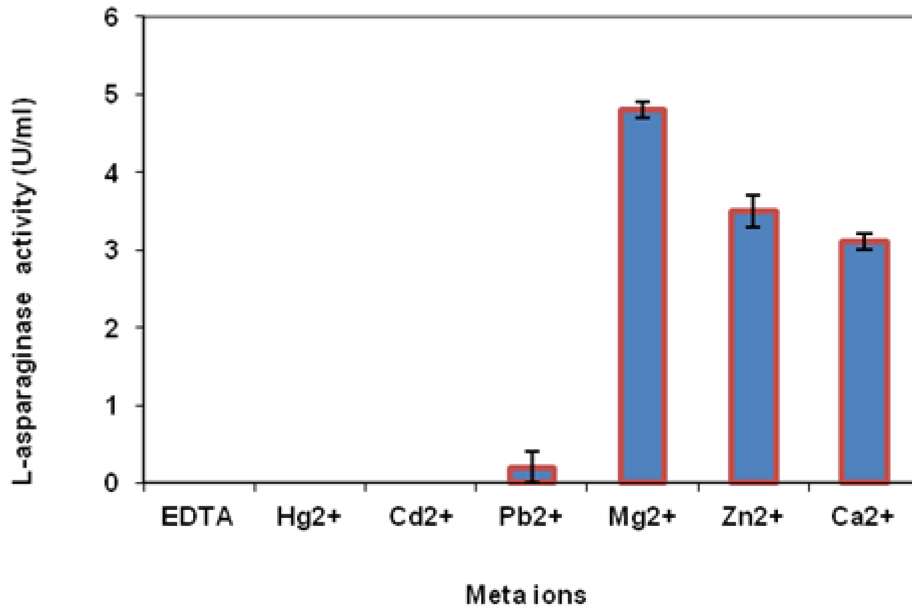

Figure 4

Activation and inhibition of some metal ions on L-Asparaginase

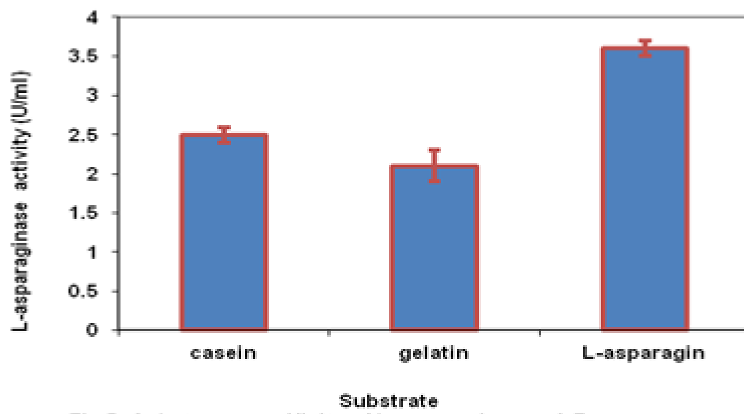

Figure 5

Maximum enzyme activity was recorded in the case of L-asparagine.

Figure 6

Km and Vmax of L-asparginase

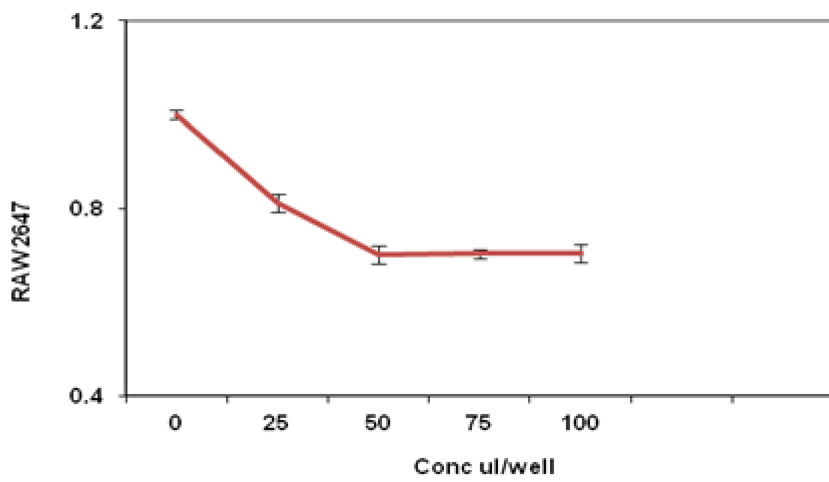

Figure 7

Antileukemic activity of L-Asparaginase