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L-Amino Acid Oxidase from Marine Bacterium: Purification, Characterization and Evaluation of its Anticancer and Antioxidant Activities

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Abstract

L-amino acid oxidases (LAAO) are extensively distributed in nature and active on most proteinogenic L-amino acids. Dehydrogenation of such amino acids produces α-keto acids and hydrogen peroxide which act as oxidative, antimicrobial and antitumor agents. In the presented work, biochemical characterization of L-amino acid oxidase from newly isolated marine bacterium *Bacillus velezensis* was performed. The molecular mass of the purified LAAO was 120 kDa which demonstrated homodimer nature with 60 kDa subunits by SDS-PAGE electrophoresis. Size exclusion chromatography was applied to achieve a purified enzyme with specific activity of 868.15 U/mg which represents 2.46-fold higher than the crude enzyme. Maximum enzyme activities were obtained at pH 8.4, 40°C while the enzyme showed a stability for 1 h over pH range 6-9 and 40°C. The values of Michalis parameters were calculated as K_m value of 0.312 mM L-Leucine with corresponding V_{max} of 19.87 U/mg. Finally, the purified LAAO was investigated an anticancer property towards different types of tumors. This objective was accomplished through measuring cytotoxicity of HepG2 and Caco-2 cancer cell lines and also through measuring the antioxidant activity. The findings indicated that LAAO individually was able to induce a cytotoxic effect by reducing the percentage of cell viability to 81% in HepG2 and Caco-2 cancer cell lines. Investigations of the antioxidant activity of LAAO showed a potent antioxidant activity. LAAO showed the ability to scavenge 1,1-diphenyl-2-pycryl-hydrazyl (DPPH) when compared with the ascorbic acid (vitamin C), a highly potent antioxidant agent. These results are promising for enzyme harnessing in different pharmaceutical and biotechnological application.

Keywords: *Bacillus velezensis*, Amino acid oxidase, L-Leucine, Purification, Characterization, Anticancer, Antioxidant.

1. Introduction

L-Amino acid oxidases (O_2-oxidoreductase, EC 1.4.3.2) are group of flavoenzymes that contain flavin adenine dinucleotide (FAD). Although FAD-dependent LAAO is the best-known group of LAAOs, another LAAO group has been reported which depends on quinone cofactor released by post-translational modification of the protein [1].

L-Amino acid oxidase enzyme catalyzes an oxidative deamination reaction of proteinogenic L-amino acids on two distinct steps. First, L-amino acid

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is oxidized to imino acid which react with water releasing ammonia and α-keto acids. Second, the reduced cofactor $FADH_2$ make a re-oxidation of the oxygen molecules to hydrogen peroxide, which makes LAAO a causative of the oxidative stress (Fig. 1) [2]. The biological effect of LAAO is mediated by either one or two ways; (a) by amino acid removal from the environment causing nutrient deficiency and/or (b) by adherence to the cell surface releasing high concentrations of hydrogen peroxide which leads to cell death [3,4].

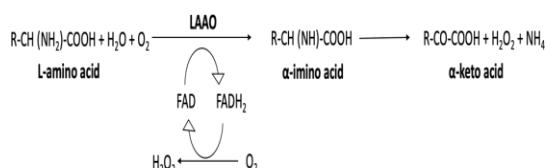

Figure 1: The catalytic mechanism of L-amino acids degradation by LAAO

LAAO has different structural and enzymatic properties depending on their producing source, physical and chemical factors such as pH and temperature, etc. [5]. For instance, the molecular mass of the enzyme is ranging between 110 and 150 kDa while its isoelectric point is in a range of 4.0 to 9.4 [6]. L- and D- amino acids are distinguished in an enzymatic asymmetric degradation of DL substrates by the microbial enzymes [7]. Most of LAAOs, specifically from bacterial origin, display a broad substrate specificity depending on the size and polarity of the substrate specificity pocket with favored hydrophobic amino acids such as L-Leu, L-Trp, L-Phe and L-Met [1,8]. However, most of FAD-dependent LAAOs are stereospecific to the L-isomer of the amino acid [9,10]. The mechanism of LAAO catalyzed reaction is similar to those reactions catalyzed by D-amino acid oxidase (DAAO) which act on the D-isomer of the substrates [11].

LAAO activity has been firstly reported by oxidation of L-tyrosine in perfused liver in 1914 [12]. Since then, they were isolated and identified from other sources such as snake venom which is the most addressed source of LAAOs [13]. Other eukaryotes and prokaryotes were recorded as LAAOs producers such as bacteria; *Bacillus carotarum*, *Rhodococcus opacus* DSM 43250, marine bacteria *Pseudoalteromonas* and fungi; *Aspergillus fumigatus*, *Trichoderma harzianum* ETS323 and *Hebeloma cylindrosporum* [7,14–18].

Owing to manifold properties of LAAOs, they are used as multifunctional enzyme in different potential applications such as detection of many diseases like autoimmune diseases and cancer [19]. It was used also as biosensors for detection of L-amino acid levels via colorimetric sensors [20,21]. As powerful biocatalyst, LAAO can be used as biocatalytic tool to de-racemise amino acids producing D-amino acids [19]. For example, Busch et al. who used LAAO as biocatalyst generating α-ketoglutarate using L-glutamate [22].

Previous studies claimed that LAAOs has a potential antimicrobial activity against different pathogenic bacteria, viruses and parasites. Thus, they act as defense barriers in different animals not only against microbes, but also against xenobiotics and predators [13]. As antitumor agent, different cytotoxic studies showed apoptotic activity of LAAO against different cancer cell lines including breast adenocarcinoma (SKBR-3), human breast adenocarcinoma (MCF-7), human cervical carcinoma (HeLa), human lung adenocarcinoma (A 549), human gastric and colon adenocarcinoma (MKN 45 and RKO), murine melanoma (B16F10), murine sarcoma 180 tumors (S180) and Ehrlich ascites tumor cells (EAT) [3,23–25]. Both antimicrobial and anticancer activities of LAAOs are because of the effect of hydrogen peroxide results from the L-amino acid oxidation. The reactive oxygen species leads to activation of caspases 3 and subsequent release of mitochondrial cytochrome C and final platelet aggregation and apoptosis [24–26].

Since the marine environment is an excellent source for different bioactive compounds producers, this paper addressed a marine bacterium *Bacillus velezensis* able to produce L-Leucine oxidase enzyme using L-Leucine as substrate. Purification and characterization of the biosynthesized enzyme as well as its practical use as anticancer compound were extensively studied.

2. Experimental

Chemicals

YPD broth medium (Yeast-Peptone-Dextrose) used for inoculum preparation was purchased from Loba Chemie Pvt. Ltd., India. L-Leucine, o-phenylenediamine (OPD), horseradish peroxidase (HRP), bovine serum albumin (BSA), Sephacryl S-300 were from Sigma-Aldrich Co. Gel filtration molecular weight marker as well as SDS molecular

weight markers were from Pharmacia Co. Other chemicals were of analytical grade.

Microorganism and Cell Lines

The microorganism of the study was previously isolates from marine beach in Alexandria (31°11′51″N 29°53′33″E) and identified as *Bacillus velezensis* using Maldi-TOF MS Identification technique.

The human hepatocellular carcinoma (HepG2) cells, Caco-2 colon cancer cell lines were collected from American Type Culture Collection (ATTC, Rockville, MD, USA). Medium, antibiotics and extras were purchased from Sigma-Aldrich, USA. The cells were grown in Roswell Park Memorial Institute Medium (RPMI 1640) with 10% Fetal Bovine Serum (FBS), 2 mM 1-glutamine and 100 U/ml (1%) penicillin/streptomycin antibiotic (100 µg/ml). Cells were kept at 37°C in humidified air comprising 5% CO_2. At 70%-80% confluence, monolayer cells have been harvested by Trypsin/EDTA.

Methods

Culture conditions

Pre-cultures were prepared by inoculation of 30 ml of YPD broth medium in 250 ml Erlenmeyer flask. The inoculated flask was then incubated for 24 h at 30°C at 200 rpm. Main production medium consists of (%): glucose; 2, $MgSO_4$; 0.05, K_2HPO_4; 0.1, KCL; 0.05, Yeast extract; 0.5 and L-Leucine; 0.5. One milliliter of the pre-culture was used to inoculate 50 ml of the production medium in 250 ml Erlenmeyer flasks. The inoculated flasks were incubated for 48 h at 30°C at 200 rpm. To extract the LAAO, cell-free extract was prepared by centrifugation a 48 h-old culture for 10 min at 15.000 Xg and 4°C (Hettich Universal, 320 R, Tuttlinger, Germany). The cell pellet was washed with sterile distilled water for three times and resuspended in one ml of 1-Butanol (Purity 99.5%, GC, Sigma Co.). The cell suspension was kept for 30 min. at 20°C under shaken conditions. Thereafter, 5 ml of sodium citrate buffer (50 mM, pH 7.0) were then added to the cell suspension which was then incubated for further 1 h at the same conditions. Finally, the cell suspension was centrifuged for 10 min at 15.000 Xg and 4°C to clarify the cell-free extract.

L-amino acid oxidase assay

The assay was carried out in duplicates in a 96-well microplate according to Kishimoto and Takahashi (2001) [27]. Briefly, 20 µl of enzyme sample was mixed with the reaction mixture (80 µl) that contained 5 mM of L-Leucine as a substrate, 0.81U/ml horseradish peroxidase (HRP) and 2 mM of O-phenylenediamine (OPD; as a substrate for peroxidase) in 50 mM of Tris–HCl buffer (pH 8.0). After incubation for 1 h at 37°C, the reaction was stopped by addition of 20 µl H_2SO_4 (2 M) The absorbance of the product (H_2O_2) was measured at 490 nm using Carry 100 UV–Vis spectrophotometer. One unit of LAAO activity was defined as the amount of enzyme which release one µmol of H_2O_2 per minute under standard assay conditions.

Purification of L-Leucine oxidase

Chromatography on Sephacryl S-300 column

Crude extract was chromatographed on column Sephacryl S-300 (142 cm X 1.75 cm i.d.). The Sephacryl S-300 column was equilibrated and run with 50 mmol L^{-1} Tris–HCl buffer (pH 8.0) in a flow rate 30 ml/h. Finally, fractions of 2 ml volume were collected.

Electrophoretic analysis

Gel electrophoresis was carried out using 7% polyacrylamide gel electrophoresis (PAGE) according to the method of Smith (1969) [28]. SDS-PAGE for the purified enzyme was conducted by 12% PAGE using the method described by Laemmli 1970) [29]. The molecular weight estimation of the purified *B. velezensis* was performed using SDS-PAGE according to Weber and Osborn (1969) [30]. For staining of proteins, the gels were stained for 2 h in 0.25% (w/v) Coomassie brilliant blue (R-250) and destained several times in the same solution lacking dye.

Protein determination

Protein concentrations were determined during the purification steps spectrophotometrically according to Bradford method (1976) [31].

Optimum pH and pH stability

To determine the optimum pH for activity of purified LAAO, the reaction mixture was incubated at different pH values (pH 7.2 to 9.0) using Tris-HCl buffer (20Mm). For the pH stability study, LAAO was pre-incubated with different buffers of different pH values (Na-acetate buffer, pH 3.6 to 5.6, Na-phosphate buffer, pH 5.7 to 7.0 and Tris-HCl buffer, pH 7.2 to 9.0) for 1 h. The remaining activity of samples was measured.

Effect of temperature on the activity and stability of LAAO

The optimum temperature for activity of purified LAAO was determined by measuring LAAO enzymatic activity at different temperature (20°-60°C). The thermal stability of the purified enzyme was studied by pre-incubating the enzyme at different temperatures (30°, 40°, 50°, and 60°C) for different

time intervals (15, 30, 45 and 60 min.) without substrate. Thereafter, the enzyme was cooled in an ice bath and the residual enzyme activity was measured.

Substrate specificity

The activity of LAAO was examined against several L-amino acids (L-Glycine, L-Alanine, L-Valine, L-Isoleucine, L-Methionine, L-Proline, L-Phenylalanine, L-Tyrosine, L-Tryptophan, L-Threonine, L-Cysteine, L-Lysine, L-Glutamine, L-Glutamate, L-Serine, L-Asparagine, L-Arginine, and L-Histidine) as substrates. Therefore, the purified enzyme was incubated separately with equal amount (5 mM) of different L-amino acids instead of L-Leu. The activity of LAAO toward L-Leucine was regarded as control (100%) and the relative activity to other L-amino acids was calculated.

Michalis-Menten constant (K_m)

The purified LAAO was incubated with raising concentrations of L-Leucine. A definite amount of LAAO was used to construct the plot for the reaction velocity (V) versus the substrate concentration [S].

Cytotoxicity assay

To assess the effect of L-Leucine Oxidase on the viability of HepG2 cells, Caco-2 cells, 3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyltetrazolium bromide, (MTT) has been used according to Hansen et al. [32]. The assay is based on the ability of the active mitochondria, in living cells, to break the tetrazolium rings (yellow) of MTT through hydrogenase enzyme, forming the formazan crystals (Dark Blue), which are insoluble and impermeable to the cell membrane. Crystals are then dissolved using acidified isopropanol and measured using a FLUO star optima multi-detection system (VWR International, Pennsylvania, USA) at 570 nm [33]. The results were expressed as the average percentages of viable cells in comparison to the untreated cells (Mean ± standard deviation). The dose-dependent curve for 24 h time point has been employed to calculate the half maximal inhibitory concentration (IC_{50}).

Antioxidant Activity

The antioxidant activity of LAAO was investigated by assessing their scavenging capacity against DPPH radicals, using the method of Gerhäuser et al. [34], as modified from van Amsterdam et al. [35]. DPPH, a deep violet radical with an unpaired electron, is used in this assay due to its ability to abstract hydrogen in presence of antioxidant radical scavenger and change its color into pale yellow. The experiment was carried out in a 96-well plate with a final volume of 200 µl. DPPH and sample aliquots at a series of concentrations ranging from 1.7 to 100 µM. DPPH solutions were used as controls which had the same serial concentrations but without the tested samples. To detect the decay in color, plates were incubated at 25°C for 30 min and then read at 520 nm. The percentage of DPPH bleaching was used to the maximal scavenging concentration (SC_{50}) compared to that of ascorbic acid.

3. Results

Purification of LAAO

The starting specific activity of L-amino acid oxidase in crude extract was 352.35 U/mg protein. One peak of LAAO was resolved from gel filtration chromatography (Fig. 2).

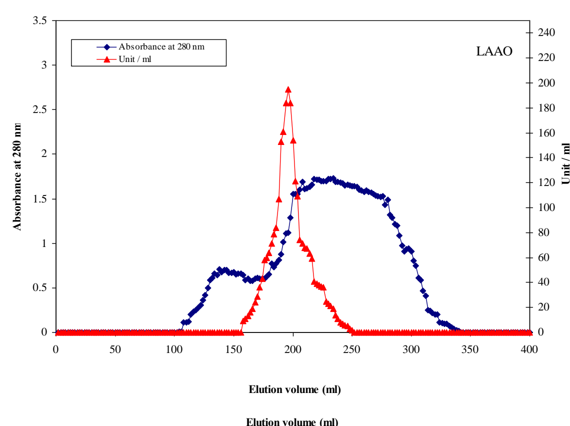

Figure 2: A chromatographic typical elution profile for LAAO crude extract on Sephacryl S-300 column (142 cm x 1.75 cm i.d.) previously equilibrated with 20 mmol L^{-1} Tris-HCl buffer, pH 8.0.

After chromatography, the LAAO specific activity was reached 868.15 U/mg protein that represents 2.463-fold with 58.07% yield (Table 1). The purified LAAO obtained after elution showed a molecular weight of 120 ± 1.43 kDa.

Table 1: A typical purification scheme of LAAO

Purification steps	Total protein (mg /ml)	Total Activity (Unit)	Specific Activity (U/mg)	Yield (%)	Fold Purification
Crude extract	11.23	3957	352.35	100.0	**1.000**
LAAO Sephacryl S-300	**2.647**	**2298**	**868.15**	**58.07**	**2.463**

One unit of LAAO activity was defined as the amount of enzyme required to produce 1 µmol of H_2O_2 per minute under the described conditions.

Electrophoretic analysis

Crude enzyme extract and samples from different purification steps were investigated by gel electrophoresis. Purity of LAAO preparation was identified by one band of protein (Fig. 3a). By comparing the electrophoretic pattern of LAAO with marker proteins on SDS-PAGE, LAAO subunit with molecular weight of 60 ± 1.25 kDa was observed. (Fig. 3b).

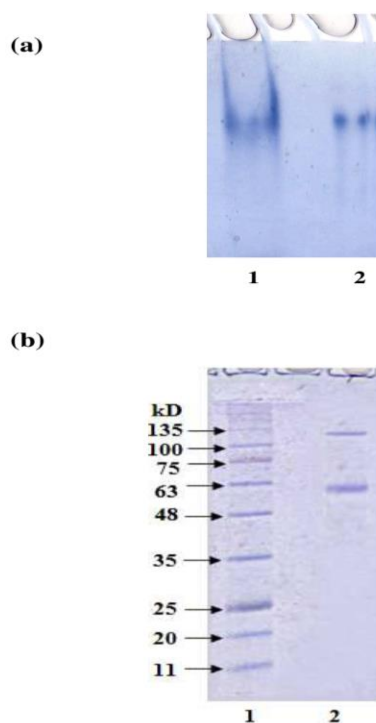

Figure 3: (a) Electrophoretic analysis of LAAO protein pattern of the different purification steps on 7% native polyacrylamide gel: (1) crude extract and (2) Sephacryl S-300 purified fraction of LAAO. (b) Subunit molecular weight determination by electrophoretic analysis of purified LAAO on 12% SDS-polyacrylamide gel: (1) molecular weight marker proteins and (2) purified LAAO.

Effect of pH

The optimum pH of purified *B. velezensis* LAAO was performed utilizing Tris-HCl buffer (20 mM) in the pH range 7.2–9.0. The highest LAAO activity, corresponds to approximately 100% relative activity was recorded at pH 8.4 (Fig. 4a). Regarding the pH stability of purified LAAO, the enzyme was incubated without substrate at different pH values (pH 3.6 to 9.0) for 1 h and then the remaining activity was estimated. The enzymatic activity showed a stability over broad pH values from 7.0 to 9.0, in contrast, the activity was decreased at pH below 7.0 (Fig 4b).

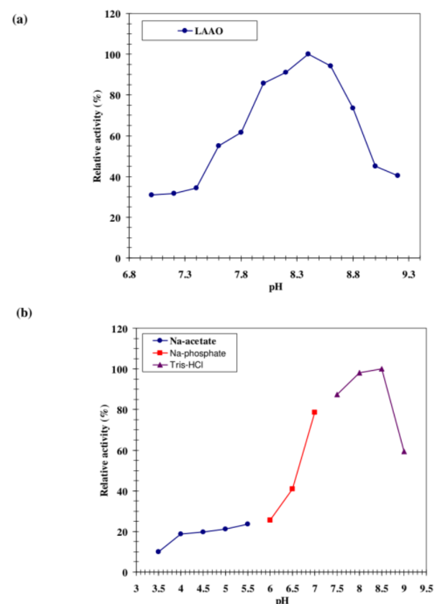

Figure 4: (a) The optimum pH for activity of purified LAAO using 20 mmol L^{-1} Tris-HCl buffer (pH 7.2 to 9.0). (b) pH stability for LAAO activity using buffer of different pH (3.6 to 9.0).

Optimum temperature and thermal stability

The optimum temperature for activity of purified LAAO was investigated through measuring LAAO enzymatic activity at different temperature (20°-60°C) (Fig 5a). The highest LAAO activity was recorded at 40°C which corresponds to 98% relative activity. To determine the thermal stability of LAAO, it was incubated at different temperatures (30°, 40°, 50°, and 60°C) for 15, 30, 45 and 60 min. without adding substrate. The LAAO activity was determined and presented in Fig 5b. LAAO activity showed a thermal stability up to 40°C with a significant decline above this degree.

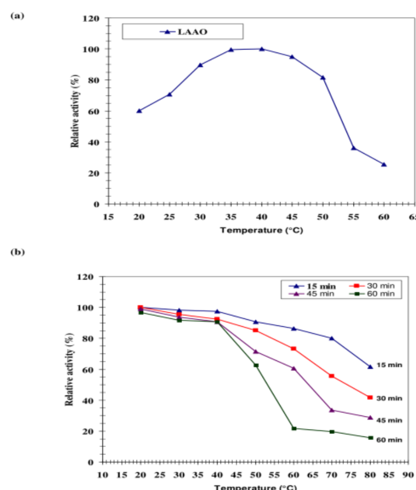

Figure 5: (a) The optimum temperature for activity of purified LAAO at different temperature (20-60 °C). (b) thermal stability of LAAO enzyme at different temperatures (30°, 40°, 50°, and 60°C) for a range of periods (15, 30, 45 and 60 min.).

Substrate affinity of LAAO

The substrate specificity of *B. velezensis* LAAO was investigated using different amino acids as substrates. The LAAO activity towards L-Leucine was regarded as 100% and the LAAO activities using other L-amino acids were calculated relatively (Table 2). Beside L-Leu, it was shown that LAAO has high affinity for both L-lys and L-Ala, respectively, while no LAAO affinity was observed with L-Thr.

Table 2: Substrate specificity of purified LAAO

Substrate (5 mM)	Relative Activity (%)
L-Leucine	100.0
L-Glycine	49.23
L-Alanine	89.73
L-Histidine	32.28
L-Tyrosine	0.00
L-Valine	43.28
L-Proline	0.00
L-Serine	85.36
L-Threonine	0.00
L-Cysteine	9.47
L-Lysine	96.88
L-Glutamine	45.78
L-Asparagine	75.67
L-Arginine	34.51
L-Tryptophan	13.68
L-Phenylalanine	46.37
L-Isoleucine	55.47
L- Methionine	12.88
L-Glutamate	15.37

Michaelis-Menten constant (K_m)

The kinetic parameters K_m and V_{max} of LAAO were investigated using L-leucine as a substrate. The K_m value of purified LAAO was found to be 0.312 mM L-Leucine while the maximum velocity (V_{max}) was calculated correspondingly as 19.87 U/mg (Fig. 6a). To emphasize, relationship between reciprocal of the reaction velocity (1/V) and substrate concentration (1/[S]) were created via a Lineweaver-Burk plot. The K_m value of LAAO was inferred as 0.312 mM L-Leucine (Fig. 6b).

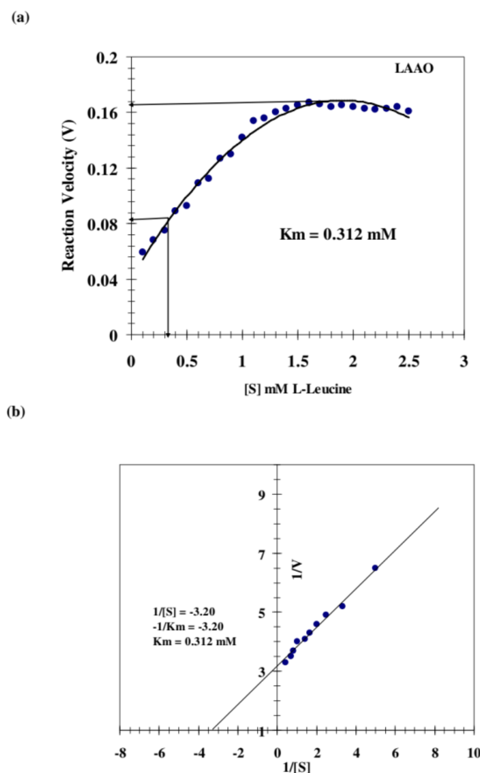

Figure 6: (a) Effect of L-Leucine concentration in mM on the reaction velocity of the purified LAAO. (b) Lineweaver-Burk plot relating the reciprocal of the reaction velocity of the purified LAAO to L-Leucine concentration.

Cytotoxicity assay

Investigation of cytotoxicity was carried out at 24 h for LAAO against HepG2 and Caco-2 cancer cells. The results revealed that LAAO individually induced a cytotoxic effect observed by reducing in percentage of cell viability using different concentrations. LAAO decreased the cell viability to 81% at a high concentration (100 µg/ml) with IC_{50} values (748.4, 362.8 µg/ml) in HepG2, Caco-2 cell lines respectively (Fig. 7). Therefore, 30% of IC_{50} can be used for the further studies.

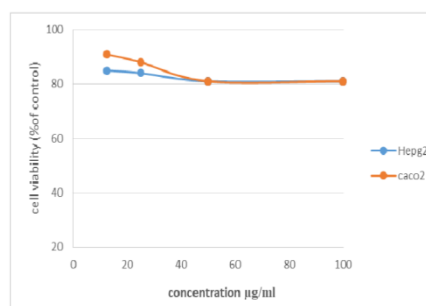

Figure 7: Cytotoxicity of LAAO as assayed by MTT method in HepG2 and Caco-2 cells after 24 h from incubation. The viability is expressed as percentage of control (mean ±SD; n=8).

Antioxidant Activity

To investigate LAAO to scavenge free radicals, a synthetic radical DPPH was used. Different concentrations of LAAO showed a promising capability to scavenge DPPH (SC_{50}=104 μM), compared to the scavenging activity of the ascorbic acid (vitamin C) (SC_{50}=95.9 μM), a highly potent antioxidant agent (Fig. 8).

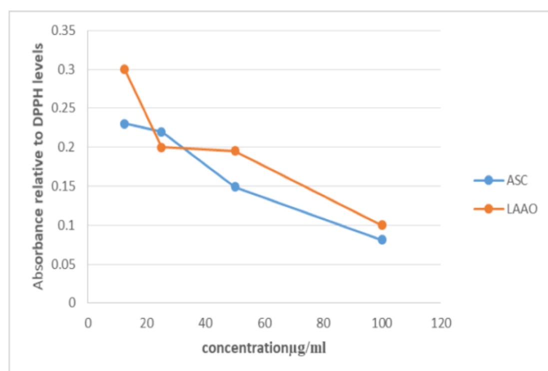

Figure 8: Antioxidant activity of Ascorbic Acid and LAAO

4. Discussion

LAAOs have a great importance for pharmacological, molecular biology, and structural studies. In the presented study, L-Leucine Oxidase was purified and characterized from *B. velezensis* using L-Leucine as substrate by consecutive one chromatographic step on Sephacryl S-300 column. The chromatographic profile obtained showed a clear fraction, recognized as LAAO.

The starting LAAO specific activity in the crude extract was 352.35 U/mg, while it was elevated to 868.15 U/mg after chromatography which represents 2.463-fold with 58.07% yield. In line, LAAO was isolated from *Aspergillus terreus* MZ769058 with specific activity 132.5 U/mg and 2.55-fold purification [6]. Purification steps samples; crude extract and Sephacryl S-300 fraction are electrophoresed on 7% native PAGE. Purity of LAAO preparation was identified as single protein band.

The molecular weight of LAAO was ascertained from the elution amount of gel filtration column to be 120 kDa. Electrophoretic analysis of the purified LAAO was compared to the protein markers on SDS-PAGE. Results demonstrated LAAO molecular weight of 120 kDa with a subunit of 60 kDa. This is in line with molecular weight of LAAO isolated from *Daboia russellii siamensis* venom [36] and from *Cerastes vipera* snake venom [37].

The optimum pH for activity of purified LAAO was found at pH 8.4 with pH steadiness at a range of pH levels of 7.0 to 9.0. In connection, optimal pH of LAAO isolated from Indian *Cobra Naja naja* is 8.5 [38]. The optimum temperature for activity of purified LAAO was recorded at 40°C with thermal stability up to 40°C, while enzymatic activity decreased in higher temperature. Similarly, optimum temperature of LAAO isolated from fungal isolate *Aspergillus terreus* MZ769058 was reported at 50°C [39].

LAAO substrate specificity study showed high affinity for L-Lys > L-Ala > L-Ser > L-Asn > L-Ile > L-Glu > L-Gln > L-Val > L-Arg > L-His > L-Gly. While low enzyme affinities to the other different L-amino acids were observed. Kinetic study of the purified LAAO showed a K_m value of 0.312 mM L-Leucine with a corresponding V_{max} of 19.87 U/mg while the K_m value of LAAO which concluded from the Lineweaver-Burk plot to be 0.312 mM L-Leucine. In line, K_m value of LAAO isolated from *Cerastes cerastes* venom was reported to be 0.67 mM [40].

LAAO showed an increased significance due to its therapeutic values as antibacterial, antiviral, antifungal and anti-HIV Properties [5]. Along these properties, LAAO also has anticancer property against different types of cell lines [41].

This current study demonstrated a cytotoxic effect of *B. velezensis* LAAO alone on Hepatocellular carcinoma HepG2 Cells and Caco-2 colon cancer cell lines in a concentration and time reliant manner. Treatment of HepG2 cells and Caco-2 colon cancer cells with LAAO led to a minimal suppression of cell proliferation with IC_{50} values (748.4, 362.8 µg/ml) respectively after 24 h of treatment. LAAO reduced the cell viability to 81% at a high concentration 100 µg/ml. Increasing the bioavailability of LAAO has been a concern for researches so as to exploit its activity in biological systems. Moreover, LAAO purified from *B. velezensis* showed a potent antioxidant activity as indicated by the high SC_{50} value (SC_{50}=104 µM) as compared to the Scavenging activity of ascorbic acid (a highly potent antioxidant agent) with the SC_{50} value of 95.9 µM.

Several studies demonstrated that LAAOs showed concentration-dependent cytotoxicity that resulted in either apoptosis or necrosis [19]. In addition, fungal L-amino-acid oxidase can induce cell death in various human tumor cell lines indicating that they may be

effective anticancer agents. In this concern, Pišlar et al. reported that LAAO isolated from *Amanita phalloides* and *Clitocybe geotropa* exhibits the highest toxicity toward Jurkat and MCF7 cancer cells in vitro and cause cell death and apoptosis in a time and concentration dependent manner [42]. The cytotoxicity of L-Lysine oxidase has been examined in vivo on various tumors, and the results proved that LAAO has a potential in treatment of patients with colorectal cancer [43]. It is well established that cell death and apoptosis mediated by LAAO is a consequence of prolonged exposure to high levels of H_2O_2 [19,42]. In addition, under extreme oxidative stress conditions the cell lysis due to the disruption of integrity of cell membrane, leading to cell shrinkage and opening of the permeability transition pores [44].

5. Conclusion

In the current study, isolated marine bacterium *Bacillus velezensis* was used as bio-factory for L-amino acid oxidase production using L-Leucine as substrate. The results of the present study demonstrated a homodimer nature of the biosynthesized LAAO with a molecular weight of 120 kDa as well as a subunit with molecular weight of 60 kDa. The purified enzyme showed approximately 2.46-fold higher specific activity than the crude enzyme. Moreover, characterization of the LAAO enzyme revealed maximum activity at pH 8.4 and 40°C. Eventually, the purified enzyme showed a potent antitumor activity against HepG2 and Caco-2 cancer cell lines with 81% reduction in the cell viability. As antioxidant agent, purified LAAO of study showed a vigorous scavenging ability to DPPH. Based on these potential findings, the biosynthesized L-amino acid oxidase shows off a promising applicability in medical and pharmaceutical industries as important antitumor and antioxidant agents.

6. Conflict of Interests

The authors declare that they have no competing interests.

7. Funding

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