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Hydroxynitrile Lyase of Wild Apricot (*Prunus armeniaca* L.): Purification, Characterization and Application in Synthesis of Enantiopure Mandelonitrile

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Abstract Hydroxynitrile lyases (HNLs) are increasingly finding application in synthesis of enantiomerically pure cyanohydrins. Cyanohydrins are important intermediates in the production of pharmaceuticals and agrochemicals. In the present studies seeds of wild apricot (*Prunus armeniaca* L.) have emerged as potential source of hydroxynitrile lyase. The HNL of wild apricot (ParsHNL) was purified 8.1 fold and 18.2 % yield with a specific activity of 141 units mg^{-1} protein. The SDS-PAGE of the enzyme revealed that it consists of subunits of 40 and 37 kDa. However, the molecular weight of holoenzyme was assessed to be 360 kDa. The enzyme showed maximum activity in 0.1 M sodium-citrate buffer having pH 4.75 at 25 °C. Thermostability studies revealed that this HNL showed activity up to 70 °C temperature and quite stable up to 50 °C. Activation energy of ParsHNL was calculated to be 37.83 kJ mol^{-1}. This enzyme has K_m of 3.76 mM, V_{max} of 188.4 μmol mg^{-1} min^{-1} and k_{cat} of 1130.4 s^{-1} using mandelonitrile as substrate while for reverse reaction using benzaldehyde as substrate it showed K_m of 16.1 mM, V_{max} of 7.21 μmol mg^{-1} min^{-1} and k_{cat} of 43.3 s^{-1}. Synthesis of mandelonitrile was carried out using ParsHNL and finally 8.88 mmole (1.184 g) of mandelonitrile was recovered which corresponded to 89 % molar conversion with 96 % ee for *R*-mandelonitrile. The yield of mandelonitrile was 411 μmol $mg^{-1}h^{-1}$. These results indicated that ParsHNL has very high potential for synthesis of cyanohydrins and can be used for the production of enantiopure cyanohydrins.

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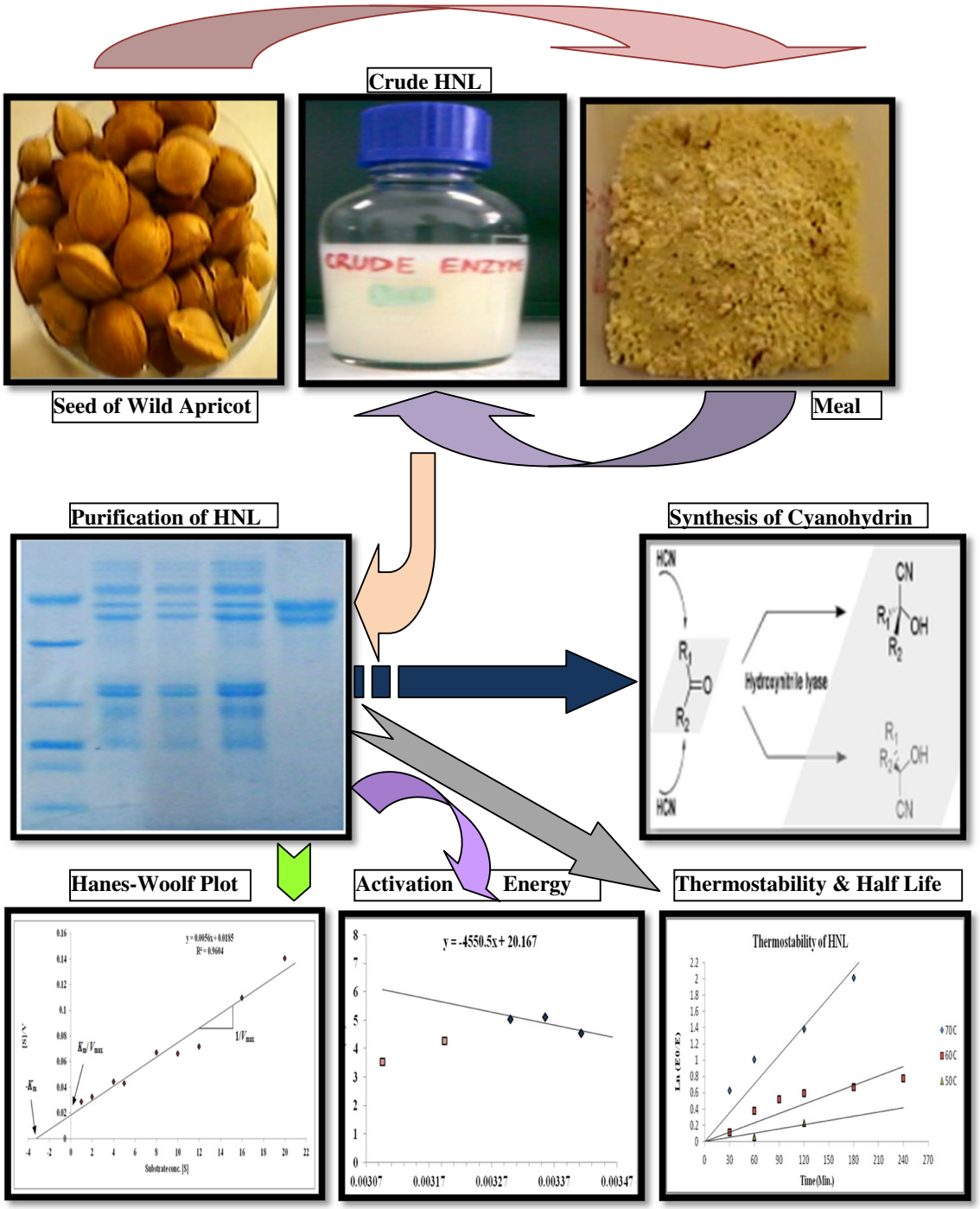
Graphical Abstract



Keywords Cyanohydrin · ParsHNL · Wild apricot · Mandelonitrile · Hydroxynitrile lyase · *Prunus armeniaca*

1 Introduction

Hydroxynitrile lyases (HNLs, EC 4.1.2.x) are the enzymes which mediate the release of hydrogen cyanide (HCN) and aldehyde or ketone from cyanohydrins [1] and catalyse enantioselective synthesis of cyanohydrins [2]. The cyanohydrins and their derivatives are finding wide applications in industries for production of pharmaceuticals, agrochemicals and cosmetics, since these can be readily converted into other synthetically relevant building blocks like α-hydroxy carboxylic acids, α-hydroxy ketones and β-amino acids [3].

The HNLs have been reported, purified and characterized from several plants e.g. almond (*Prunus amygdalus*), flax (*Linum usitatissimum*), cherry (*Prunus serotina*), peach (*Prunus persica*), capulin (*Prunus capuli*), rosary pea (*Abrus precatorius*), rubber tree (*Hevea brasiliensis*), cherrylaurel (*Prunus laurocerasus*), cassava (*Manihot esculenta*), Japanese apricot (*Prunus mume*), *Prunus lyonii*, *Phlebodium aureum*, *Ximenia americana*, *Eriobotrya japonica*, *Passiflora edulis*, etc. [1, 4–17].

In the present investigation, purification and characterization of HNL from seeds of wild apricot (*Prunus armeniaca* L.) (ParsHNL) was undertaken because the seeds are by-product of apricot processing industry. Use of these seeds as a source of enzyme is an economically and ecologically interesting alternative to their existing processing and utilization. The seeds of *P. armeniaca* have been shown to contain hydroxynitrile lyase and β-glucosidase, but have not been explored so far. Though a crude HNL preparation from a cultivated variety of *P. armeniaca* i.e. white apricot (shakarpara) (which is more juicy but less acidic than wild apricot) has been used for synthesis of several cyanohydrins [18], but no study has been carried out on purification and characterization of HNL of *P. armeniaca*. Therefore, in the present studies an attempt has been made to purify and characterize ParsHNL and to use it for the synthesis of mandelonitrile.

2 Materials and Methods

2.1 Plant Material

Seeds of wild apricot (*P. armeniaca*) used as source of HNL were obtained from the Department of Food Science and Technology, Dr. Y S Parmar University of Horticulture and Forestry, Solan, India.

2.2 Preparation of Crude Enzyme Extract

Crude hydroxynitrile lyase was extracted from the kernel of *P. armeniaca* seeds according to method outlined by Han et al. [19] with slight modification. In brief, the seeds were soaked in distilled water for 24 h at room temperature, washed with distilled water and ground with chilled ethyl acetate. It was air dried and stored at 4 °C and termed as meal. The crude HNL was prepared by suspending this meal in distilled water (7.4 g/100 mL), its pH adjusted to 7.4 with 1 N NH_4OH (for effective extraction of enzyme) and was kept at 4 °C overnight. It was centrifuged at 15,000×*g* for 15 min and supernatant was collected and its pH was adjusted to 5.5 with 50 % acetic acid. It was filtered and the filtrate was used as crude enzyme in subsequent experiments. The protein concentration in the enzyme preparation was determined following the method of Bradford [20].

2.3 Assay of Hydroxynitrile Lyase Activity

The activity of HNL was assayed according to the method of Siritunga et al. [21] in 1 ml reaction mixture comprising 0.1 M sodium citrate buffer (pH 5.0) containing 10 µmoles of mandelonitrile and 5 µl crude enzyme (3 µg protein), at 25 °C in water bath for 30 min, against a control reaction (without enzyme). The liberated cyanide was estimated colourimetrically at 585 nm wavelength and one unit of enzyme activity was defined as µmole of cyanide formed min^{-1} mg^{-1} of protein.

2.4 Purification of ParsHNL

All steps of protein purification were performed at 4 °C using 0.1 M sodium citrate buffer (pH 5.0) and centrifugation was carried out at 15,000×*g* for 15 min at 4 °C. The crude enzyme was prepared from 1.42 g meal of wild apricot following the procedure described in Sect. 2.2.

2.4.1 Ammonium Sulphate Precipitation

Ammonium sulphate fractionation of crude enzyme preparation (12 mg crude enzyme protein in 20 ml buffer) was done to concentrate HNL activity.

2.4.2 Gel Permeation Chromatography

The gel permeation chromatography was carried out using Sephacryl S-100 column. Dialysed sample was applied on to the pre-equilibrated column with start buffer (0.1 M sodium citrate buffer pH 5.0), eluted with elution buffer (0.1 M sodium citrate buffer pH 5.0 containing 0.1 M NaCl) with flow rate of 30 ml/h and fractions of 3 ml were collected. The fractions rich in HNL activity were pooled, analysed and termed as GPC HNL preparation.

2.4.3 Ion Exchange Chromatography

The ion exchange chromatography was performed with DEAE-Sepharose anion exchanger column. The GPC HNL preparation was applied on to the pre-equilibrated column, which was eluted with start buffer (0.1 M sodium citrate buffer pH 5.0). Fractions of 1 ml each were collected with a flow rate of 60 ml/h and the protein fractions rich in HNL activity were pooled and analysed electrophoretically. The enzyme preparation at this stage was designated as IEC.

2.4.4 Sodium Dodicyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The enzyme preparations of various stages of purification were subjected to SDS-PAGE using the procedure of Laemmli [22] (Fig. 1) . The molecular mass of the native enzyme was determined by gel-filtration chromatography on a Sephacryl S-300 column HR 16/60 (GE Healthcare) by comparing the elusion volume of reference proteins markers (Fig. 2).

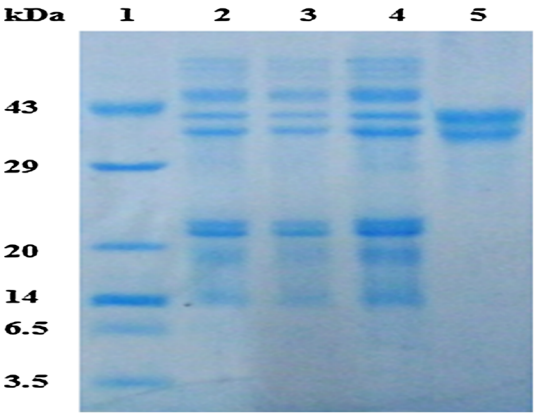


Fig. 1 SDS-PAGE of purified ParsHNL. *Lane 1* was loaded with molecular mass standard: ovalbumin (43 kDa), carbonic anhydrase (29 kDa), soyabeen trypsin inhibitor (20 kDa), lysozyme (14.3 kDa), aprotinin (6.5 kDa) and insulin chain b (3.5 kDa). Crude enzyme (*lane 2–4*) and purified ParsHNL (*lane 5*)

2.4.5 Determination of FAD of ParsHNL

The purified enzyme was denatured by heating in boiling water bath for 10 min and FAD was estimated after filteration by HPLC in supernatant using 70 % acetonitrile in water as mobile phase using 515 HPLC pump (Waters) equipped with SunFire C18 5 µm (4.6 × 250 mm) column (Waters) and 2998 Photodiode Array Detector (Waters) with flow rate of 60 ml/h. The absorption was taken at 263 nm.

2.5 Optimization of Reaction Conditions for Assay of ParsHNL

Various parameters were optimized to determine ideal reaction conditions for assay of ParsHNL activity. The effect of substrate concentration on ParsHNL activity was also studied using mandelonitrile as substrate. The activity of the purified ParsHNL was determined at different pH (3.5–6.5) using several buffer systems [sodium citrate, sodium acetate, sodium phosphate, potassium phosphate and citrate phosphate] of molarity 0.1 M using mandelonitrile as substrate. Effect of temperature (ranging from 20 to 50 °C) was studied on the activity of purified ParsHNL, to determine optimum reaction temperature. The activation energy of the enzyme was also determined. Effect of various metal ions (at 10 mM conc.) on ParsHNL activity was also evaluated (Table 2).

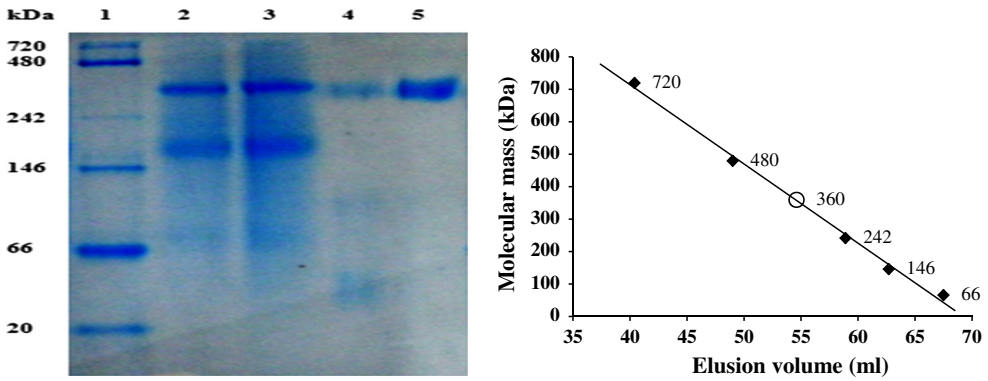


Fig. 2 Non-denaturating PAGE of purified ParsHNL and determination of molecular mass. *Lane 1* was loaded with molecular mass standard: apoferritin band 1 (720 kDa), apoferritin band 2 (480 kDa), β-phycoerythrin (242 kDa), lactate dehydrogenase (146 kDa), bovine serum albumin (66 kDa) and soybean trypsin inhibitor (20 kDa). Crude enzyme (*lane 2–3*), gel permeation chromatography (*lane 4*) and purified ParsHNL (*lane 5*)

2.6 Thermostability and Determination of Half-Life of the Enzyme

For determination of half-life of the enzyme and its thermostability, it was pre-incubated at different temperature (25, 30, 50, 60 and 70 °C) up to 72 h and its activity was determined at regular interval of time. In order to assess the denaturation of ParsHNL, its activity reduction with time was expressed as:

$$\frac{d[E]}{dt} = -k_d \times [E] \quad (1)$$

where [E] denotes HNL activity at time t min; k_d the first-order enzyme deactivation constants of HNL s^{-1}. Integrating Eq. 1, we obtain:

$$[E] = [Eo]e^{-kdt} \quad (2)$$

where [Eo] represents original HNL activity at t = 0. Solving for ln ([Eo]/[E]) from Eq. 2, we obtain:

$$\ln\left(\frac{Eo}{E}\right) = k_d \times t \quad (3)$$

From Eq. 3, it has been found that ln ([Eo]/[E]) changes linearly with thermal duration t with a slope of k_d. The half-life of ParsHNL ($t_{1/2}$), was derived from Eq. 4 as following:

$$t_{1/2} = \frac{0.693}{k_d} \quad (4)$$

2.7 Determination of K_m and V_{max} of ParsHNL

Hyperbolically fitted plots drawn between V and [S] were used to determine various kinetic parameters (K_m, V_{max}, k_{cat}, k_{cat}/K_m and K_m/V_{max}) of purified ParsHNL using mandelonitrile as substrate (1–20 mM, 25 °C) for catalysis of mandelonitrile into benzaldehyde and HCN, as well as for determining the kinetic parameters for synthesis reaction of ParsHNL using benzaldehyde as substrate (4–16 mM).

2.8 Synthesis of Mandelonitrile Using ParsHNL

The procedure of Zandbergen et al. [23] was used for synthesis of mandelonitrile using purified ParsHNL with slight modification. In a flat bottom flask, 650 mg of KCN (in ethyl acetate) and 1.016 ml benzaldehyde (10 mmole; in DMSO) with 1.35 mg (190.3 units) of ParsHNL were added to it and final volume was made to 100 ml with ethyl acetate. The reaction was continued while stirring for 16 h at 4 °C. Mandelonitrile synthesized in the reaction was quantified by HPLC using 65 % acetonitrile in water as mobile phase using 515 HPLC pump (Waters) equipped with SunFire C18 5 µm (4.6 X 250 mm) column (Waters) and 2998 Photodiode Array Detector (Waters) with flow rate of 60 ml/h. To determine the enantiomeric excess (ee %), chiral chromatography was performed using Chiralcel-OJH 5 µm (4.6 × 250 mm) column (Daicel) and SPD-10A vp UV detector, with mobile phase of *n*-hexane/isopropanol in ratio of 80/20 respectively with flow rate of 60 ml/h. Absorbance was recorded at 210 nm and data analysed using Shimadzo LC solution software. The % ee was calculated as $\frac{R-S}{R+S} \times 100$. A blank reaction was also performed without enzyme and the amount of mandelonitrile formed in blank was substracted from enzyme catalysed reaction.

3 Results and Discussion

3.1 Extraction of Crude Enzyme

12 mg crude HNL protein with 209 units (specific activity 17.4 U/mg protein) could be prepared from 1.42 g meal. Earlier Pratush et al. [8] were able to extract 24.32 mg of crude HNL protein (64 U/mg) from 11.1 g meal of rosary pea while Techawaree et al. [16] extracted 1000 mg of crude HNL protein using 2 kg seeds of *Eriobotrya japonica* with specific activity of 0.8 U/mg of protein. The recovery of protein was found to be 0.84, 0.22 and 0.05 (% mg protein obtained from per mg meal/seed used) for wild apricot, rosary pea and *Eriobotrya japonica* respectively. It reflects either the procedure followed was efficient or the material used i.e. seed of wild apricot are rich in HNL.

3.2 Purification of ParsHNL

The purification of ParsHNL was carried out by using ammonium sulphate precipitation, gel filtration and ion exchange chromatographies. The maximum increase in specific activity (45 U/mg protein) was observed in 30–40 % ammonium sulphate saturation with a yield of 50.8 % and 2.6 fold of purification (Table 1). The gel permeation chromatography was done and the fraction number 9 and 10 showed high HNL activity, showing 4.9 fold purification with 31.3 % yield of ParsHNL. The DEAE Sepharose ion exchange chromatography fractions numbering 2 to 6 having high HNL activity were pooled and this exhibited 8.1 fold purification with a yield of 18.2 % of the enzyme (Table 1). Previously, 4.3 fold purification with 60 % yield in *Prunus lyonii* [13], 122 fold purification with 38 % yield in *Ximenia americana* [15], 1.71 fold purification with 50 % yield in *Linum usitatissimum* [4, 24], 49 fold purification with 36 % yield in *Eriobotrya japonica* [16] and 9 fold purification with 26 % yield in rosary pea [8] had been reported. Most of the HNL

Table 1 Purification of ParsHNL

Stage of purification	Total protein (mg)	Specific activity (U/mg protein)	Total activity (units)	Yield (%)	Purification (fold)
Crude enzyme	12	17.4	209	100	1
ASF	2.36	45	106.2	50.8	2.6
GPC	0.77	85	65.4	31.3	4.9
IEC	0.27	141	38.1	18.2	8.1

Specific activity = One unit of enzyme activity was defined as μmole of cyanide formed min^{-1} mg^{-1} of protein

ASF Ammonium sulphate fraction; *IEC* Ion exchange chromatography; *GPC* Gel permeation chromatography

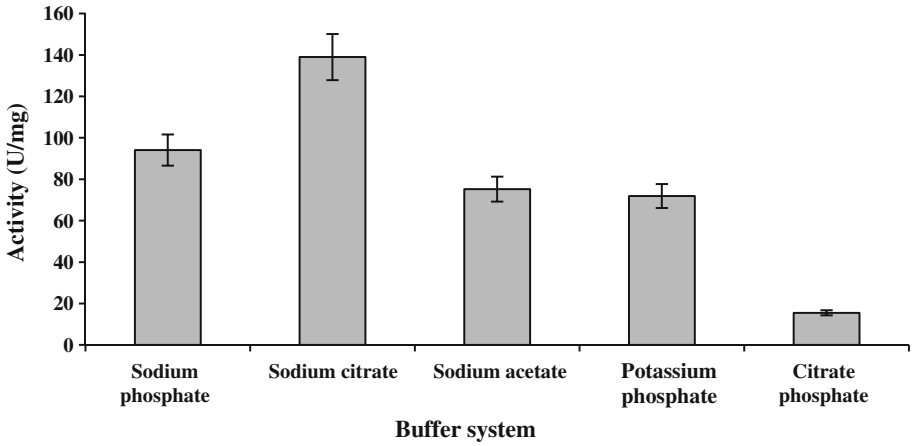


Fig. 3 Effect of buffer system on ParsHNL activity. The activity of HNL was estimated with various buffers system with salinity of 0.1 M

of Rosaceace family contains FAD as co-factor, hence possibilities of the presence of FAD as cofactor of ParsHNL was investigated. It was observed that ParsHNL is FAD containing enzyme, as clear peak of FAD was observed in supernatant corresponding to FAD standard. From the enzyme solution (3.2 mg ml^{-1}) the observed FAD concentration was found to be 77.6 mM in supernatant after denaturation.

The electrophoretic analysis of the purified ParsHNL revealed that its molecular mass was approximately 360 kDa (as estimated by gel permeation chromatography as well as Native-PAGE; Fig. 2) consisting of heteromeric units having size of 40 and 37 kDa subunits (as revealed by SDS-PAGE; Fig. 1). Earlier Siriporn and Montri [25] have reported homotetramer of HNL with subunit molecular weight of 25.6 kDa in cassava while HNL of *Eriobotrya japonica* has a molecular size of 72 kDa [16]. Pratush et al. [8] have purified HNL of rosary pea having a molecular mass of 205 kDa comprising of two types of subunits with molecular mass of 42 and 36.5 kDa.

3.3 Characterization of purified ParsHNL

The different reaction conditions like buffer system and pH were optimized for the purified ParsHNL. In order to select a suitable buffer system for assay of ParsHNL, its activity in sodium citrate (139 U/mg protein), sodium acetate (75.2 U/mg protein), sodium phosphate (94.1 U/mg protein), potassium phosphate (71.9 U/mg protein) and citrate phosphate (15.5 U/mg protein) buffers (each having salinity of 0.1 M) was estimated and sodium citrate buffer of molarity 0.1 M turned out to be best for HNL activity (Fig. 3). Effect of substrate (mandelonitrile) concentration was studied and it was observed that ParsHNL showed highest activity at 12 mM reaction mixture (Fig. 7). ParsHNL showed optimum activity at pH 4.75–5.0 with mandelonitrile (Fig. 4) in 0.1 M sodium citrate buffer. Among the metal ions tested, mercuric ion (Hg^{2+}) completely inhibited the activity of ParsHNL. This suggest, that the ParsHNL has cysteine residue at its active site which is also supported by the crystal structure of HNL from *P. dulcis* (1ju2) [26]. Other metal ions i.e. Sr^{2+}, Cs^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+} and Pb^{2+} showed partial inhibition of HNL activity and the rest had either very less or no effect on activity of ParsHNL (Table 2).

3.4 Effect of Temperature on ParsHNL Activity

HNL activity was assayed at different temperature range (20–50 °C) and 25 °C turned out to be an optimum temperature for the assay of this enzyme (Fig. 5). The increase in incubation temperature beyond 30 °C resulted in significant loss in the enzyme activity. Initial rate of reaction at different temperatures plotted in Arrhenius format [Ln

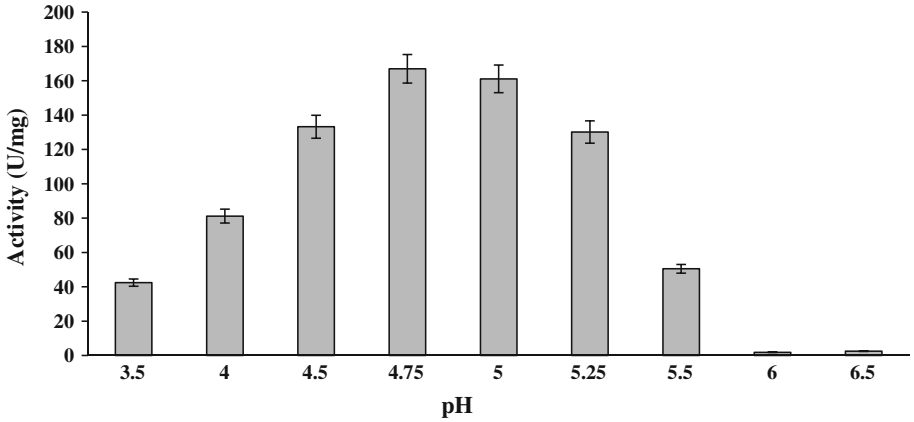


Fig. 4 Effect of pH on ParsHNL activity. The activity of HNL was estimated at various pH ranging from 3.5 to 6.5

Table 2 Effect of various metal ions on ParsHNL

Metal ion	% Residual activity
Control	100
Strontium (Sr^{+2})	84.6
Cesium (Cs^{+2})	89.3
Tungsten (W^{+6})	95.3
Cobalt (Co^{+2})	78.2
Copper (Cu^{+2})	78.9
Magnesium (Mg^{+2})	90.0
Ferrous (Fe^{+2})	70.7
Ferric (Fe^{+3})	77.8
Manganese (Mn^{+2})	83.0
Cadmium (Cd^{+2})	96.6
Zinc (Zn^{+2})	98.0
Calcium (Ca^{+2})	99.0
Lead (Pb^{+2})	62.7
Mercury (Hg^{+2})	0.0

(k) vs. 1/T] yielded a linear profile from which activation energy of 37.83 kJ/mol was calculated which showed high catalytic efficiency of the enzyme. Earlier Xu et al. [27] reported activation energy of MeHNL-catalyzed transcyanation of acetyltrimethylsilane (ATMS) and its carbon analogue 3,3-dimethyl-2-butanone (DMBO) with acetone cyanohydrins and found activation energy of 37.0 and 49.0 kJ/mol for ATMS and DMBO respectively.

3.5 Thermostability and Determination of the Half-Life of the ParsHNL

Based on the first-order enzyme deactivation assumption and Eq. 3, the ParsHNL deactivation rate coefficient k_d at 25, 30, 50, 60 and 70 °C are 0.89×10^{-6}, 1.36×10^{-6}, 2.38×10^{-5}, 7.67×10^{-5} and 25.19×10^{-5} s^{-1} respectively (Table 3), and their corresponding half-life ($t_{1/2)}$ are 216, 141, 8.1, 2.5 and 0.77 h (Fig. 6). ParsHNL does not show any significant loss of activity at 4 °C, hence it has good potential for industrial application.

3.6 K_m and V_{max} of ParsHNL

Characterization of an enzyme for its kenetic parameters is important in view of its industrial applicability. ParsHNL showed V_{max} of 188.4 µmol $min^{-1}mg^{-1}$ protein, K_m of 3.76 mM, k_{cat} of 1130.4 s^{-1}, k_{cat}/K_m of 300.6 $mM^{-1}s^{-1}$

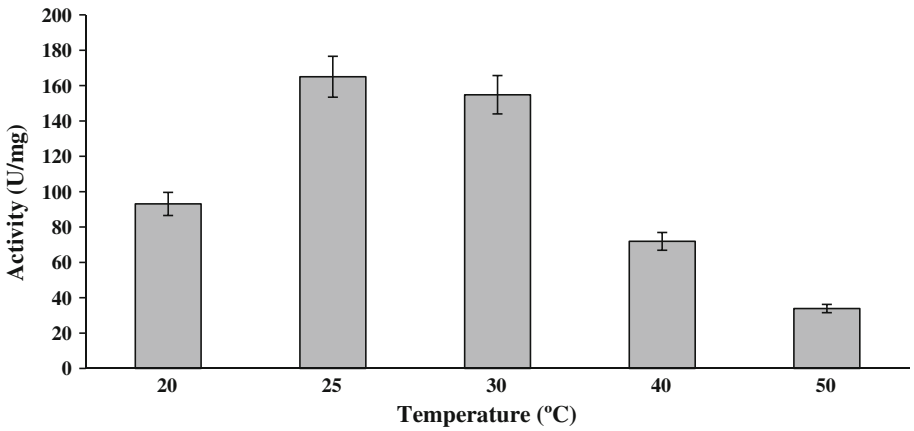


Fig. 5 Effect of temperature on ParsHNL activity. The activity of HNL was determined at various temperatures ranging from 20 to 50 °C

Table 3 Half-life and deactivation constant (k_d) of ParsHNL

Sl. No.	Temperature (°C)	k_d (s^{-1})	$t_{1/2}$ (h)
1	70	25.19×10^{-5}	0.77
2	60	7.67×10^{-5}	2.5
3	50	2.38×10^{-5}	8.1
4	30	1.36×10^{-6}	141
5	25	0.89×10^{-6}	216

and K_m/V_{max} of 0.02 mM μmol^{-1}min mg with mandelonitrile as substrate (Fig. 7; Table 4). The observed values of kinetic parameters with benzaldehyde as substrate were V_{max} of 7.21 μmol min^{-1}mg^{-1} protein, K_m of 16.07 mM, k_{cat} of 43.3 s^{-1}, k_{cat}/K_m of 2.7 mM^{-1}s^{-1} and K_m/V_{max} of 2.23 mM μmol^{-1} min mg (Fig. 8; Table 4), whereas K_m and V_{max} of HNL reported from different sources i.e. *Phlebodium aureum* [14], *Prunus lyonii* [13] and *Linum usitatissum* [4, 24] were 0.83 mM benzaldehyde and 60.1 μmol min^{-1} mg^{-1} protein, 93 mM mandelonitrile and 450 μmol min^{-1} mg^{-1} of protein, and 2.5 mM benzaldehyde and 1.11 μmol min^{-1} mg^{-1} of protein respectively. These kinetic parameters exhibited that ParsHNL has good affinity for substrate and is among one of the very active HNLs reported till date.

3.7 Synthesis of Mandelonitrile Using ParsHNL

The potential of this enzyme to synthesize mandelonitrile (cyanohydrins) in reverse reaction was explored. In the reaction mixture 10 mmole (1.016 ml) of benzaldehyde

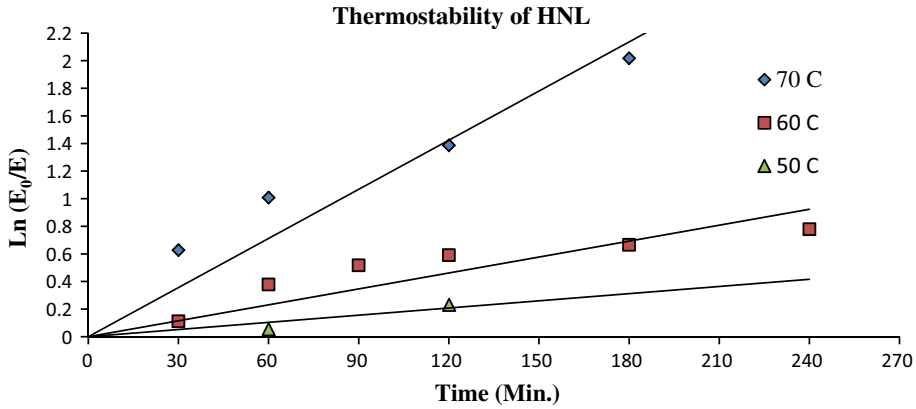


Fig. 6 Determination of deactivation rate coefficient (k_d) and half-life ($t_{1/2}$) of ParsHNL. The ln of (E_O/E) was plotted against incubation time of enzyme at various temperatures

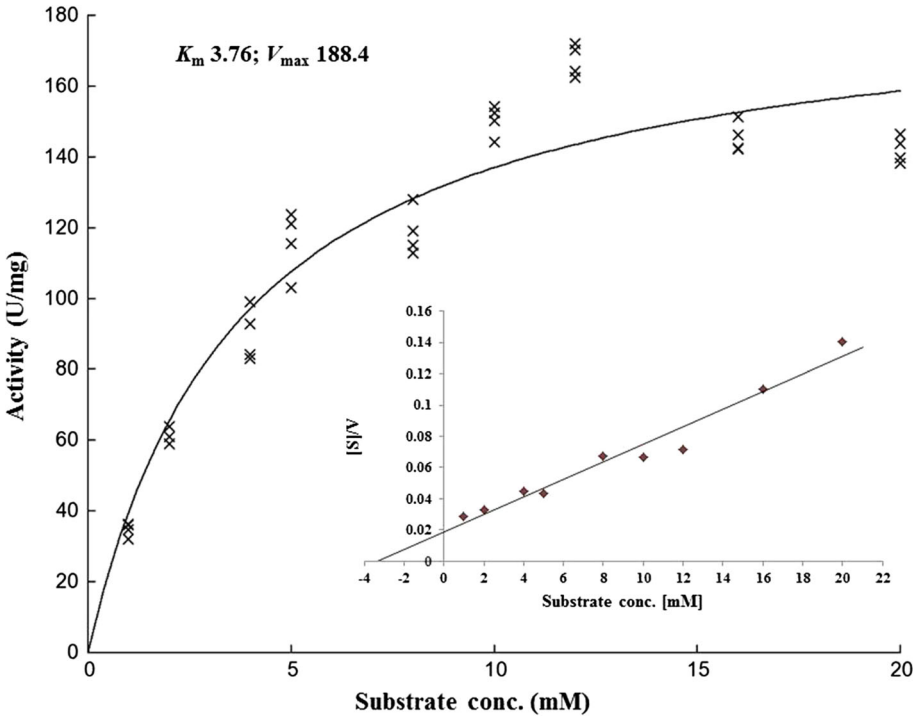


Fig. 7 Hyperbolically fit V vs. [S] plot. Kinetic parameters of purified ParsHNL were determined using mandelonitrile as substrate

Table 4 Kinetic properties of ParsHNL and some other HNLs

Sl. no	Substrate	V_{max}	K_m	k_{cat}	k_{cat}/K_m	K_m/V_{max}	Source	Reference
1	Benzaldehyde	7.21	16.1	43.3	2.7	2.23	*P. armeniaca*	Present study
2	Mandelonitrile	188.4	3.76	1130.4	300.6	0.02	*P. armeniaca*	Present study
3	Benzaldehyde	60.1	0.83	–	–	0.014	*Phlebodium aureum*	[14]
4	Mandelonitrile	450	93	–	–	0.207	*P. lyonii*	[13]
5	Benzaldehyde	1.11	2.5	–	–	2.25	*Linum usitatissum*	[4, 24]
6	Mandelonitrile	625	13	2135.4	164.3	0.021	*Abrus precatorious*	[8]
7	Mandelonitrile	197	0.38	328.3	864	0.002	*P. amygdalus turcomanica* Lincz.	[29]
8	Benzaldehyde	2.7	14	4.5	0.32	5.18	*P. amygdalus turcomanica* Lincz.	[29]
9	Mandelonitrile	46.5	0.161	56	348	0.003	*Eriobotrya japonica*	[16]

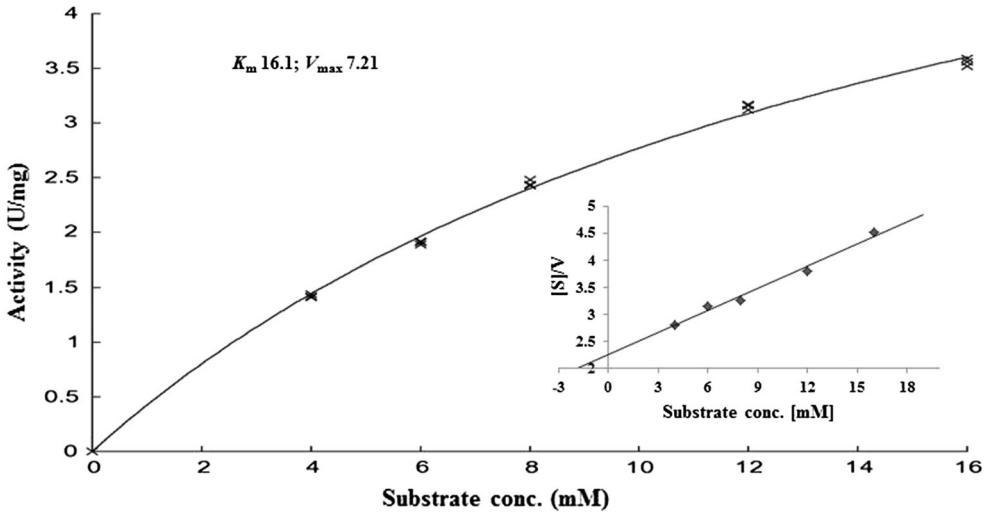


Fig. 8 Hyperbolically fit V vs. [S] plot. Reverse kinetic parameters of purified ParsHNL were determined for synthesis of mandelonitrile using benzaldehyde as substrate

and 10 mmole of potassium cyanide were used in 100 ml reaction mixture and finally 8.88 mmole (1.184 g) of mandelonitrile was recovered which corresponded to 89 % molar conversion. The yield of mandelonitrile was 411 µmol mg^{-1} h^{-1}. The ParsHNL synthesizes (*R*)-mandelonitrile preferencialy as observed in chiral HPLC with 96 % ee. The percentage molar conversion rate was much higher as compared to previously reported HNLs e.g. 73 % in case of HNL of *Prunus capuli* and 61 % molar conversion by HNL of *Mammca americana* [7] and was comparable to HNL of *Abrus precatorius* i.e. 90 % [8], almond (*Prunus amygdalus*; 99 % ee and 100 % conversion), plum (*P. domestica*; 99 % ee and 92 % conversion) and peach (*P. persica*; 99 % ee and 94 % conversion) [28].

4 Conclusion

Present study revealed that wild apricot (*P. armeniaca*) is a good source of HNL for enantioselective synthesis of cyanohydrins. Wild apricot contains much more amount of HNL as compared to other sources and thus it could be beneficial for the pharmaceutical, agrochemicals and cosmetics industries due to its cost effectiveness. The stability studies revealed that ParsHNL could be succesfully used for the industrial uses without any significant loss of activity at its operational temperature. The results of various biochemical and kinetic properties (K_m and V_{max}) alongwith synthesis of mandelonitrile indicated that ParsHNL is (*R*)-selective HNL and has high potential for enantiopure synthesis of *R*-cyanohydrins.

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