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RESEARCH ARTICLE

ENZYMATIC DE-RHAMNOSYLATION OF ORANGE PEEL NARINGIN BY 1 α -L-RHAMNOSIDASE FROM *ASPERGILLUS TERREUS* MTCC-3374

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ABSTRACT

α-L-rhamnosidases cleaves terminal α-L-rhamnose specifically from a number of natural glycosides. Most of α-L-rhamnosidases reported so far have their pH optima in the acidic pH range. α-L-rhamnosidases with different physicochemical properties are suitable for different applications. Therefore, there is a scientific need to identify different sources of α-L-rhamnosidases with different properties suitable for different applications. The α-L-rhamnosidase has wide occurrence in nature and has been reported from animal tissues, plants, yeasts, fungi and bacteria. In this study we reports α-L-rhamnosidase secreted *by Aspergillus terreus* MTCC-3374 are potential catalysis in hydrolysis of naringin content present in orange peels. α-L-rhamnosidase from the culture filtrate of a fungal strain, *Aspergillus terreus* MTCC-3374 has been purified to homogeneity. The procedure involved concentration by ultrafiltration and anion-exchange chromatography on DEAE. The purified enzyme gave 16 fold purification with 30% recovery of the activity correspond a single protein band to molecular mass of 79.0 kDa in SDS-PAGE analysis. The native PAGE analysis of the purified enzyme also gave single protein band confirming the purity of the enzyme preparation. The K_m and V_{max} value of the enzyme were 1.5 mM and 34.5 μmole/min/mg, respectively, using p-nitrophenyl α-L-rhamnopyranoside as the substrate. The k_{cat} value was 31.03 s^{-1} giving k_{cat}/K_m value of 2.07 $\times 10^4$ $M^{-1}s^{-1}$. The pH and temperature optima of the enzyme were 3.5 and 55.0°C, respectively. The purified enzyme preparation successfully hydrolyzed orange peel naringin in to de rhamnosylated product prunin.

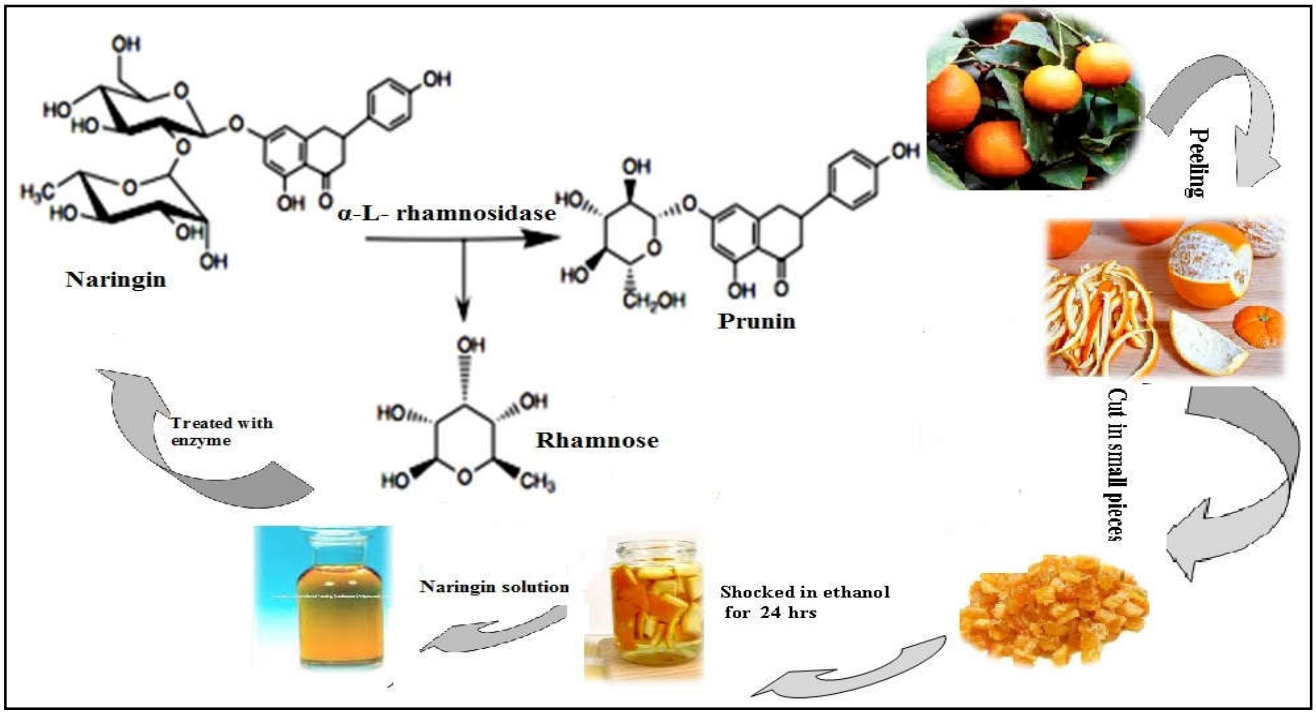
Citation: Sarita Yadav and Sudha Yadava, **2019.** "Enzymatic de-rhamnosylation of orange peel naringin by α-L-rhamnosidase from *Aspergillus terreus* MTCC-3374", *International Journal of Current Research*, 11, (07), 5587-5594.

INTRODUCTION

Biocatalysis represents a valuable tool for industrial biotechnologies. The use of enzymes as biocatalyst can have significant benefits compared to conventional chemical technologies, for achieving high reaction selectivity, high reaction rate improved product purity and decrease in chemical waste production (Viviana *et al.,* 2014, Xoo *et al.,* 2010). Enzymes are degradable in nature and environmental friendly and help to reduce production cost. All these factor adds to their advantage as compare to chemical processes (Machiulsen *et al.,* 2007). The natural source of enzyme include plant and animal sources, microbial source are preferred due to economic as well as other technical benefits such as higher yields obtained within the shortest fermentation time. Different microbial source such as bacteria, yeast and fungi have been produced enzyme by utilizing different substrate such as starch, glucose and other inorganic salt (Aunstrup *et al.,* 1974). The citrus fruit juice processing industry generates excessive volume of agro Industrial wastes. Being rich in nutrients these waste can used as raw materials for production of enzyme as well as valuable pharmaceutical compounds. Citrus fruit peel contains two main compounds they are limonin and naringin.

This study depends on the naringin content in orange fruit peel which hydrolyzed by enzyme α-L-rhamnosidase secreted by fungal strain *Aspergillus terreus* MTCC-3374. Naringin (4',5,7-trihydroxyflavanone-7-rhamnoglycoside) is a flavonoid glucoside and it is one of the main bitter compound in citrus fruits were hydrolyzed by α-L-rhamnosidase to prunin which is 33% as bitter as naringin and L-rhamnose (Iness and Ibrahim 2013) . α-L-rhamnosidase [EC.3.2.1.40] are a class of glycosyl hydrolases that specificcaly cleave terminal α-L-rhamnose from a large number of flavonoids, polysaccharides, steroids, glycopeptides antibiotics and glycolipids (Igbonekwu *et al.,* 2018, Monika *et al.,* 2013). The enzyme have been found in some plants and animal tissues as well as in yeasts, fungi and bacteria(Puri et. al.2000, Yadav *et al.,* 2010). The enzyme has several biotechnological applications such as debittering of citrus fruit juices (Park *et al.,* 2005, Hashimato *et al.,* 1999) hydrolysis of hesperidin to release hesperetin glucoside which is an important precursor in sweetener production (You *et al.,* 2010), preparation of prunin (antiviral, anti-inflammatory activity and used as sweetening agent of diabetics) from naringin (Deniel *et al.,* 1990), preparation of isoquercetin which has protective effects against the development of diabetics (Yadav *et al.,* 20017), and L- rhamnose, a chiral

intermediate in organic synthesis and used as pharmaceuticals and plant protecting agents (Yadav *et al.,* 2012). α-L-rhamnosidase have recently been the focus of an increasing scientific interest. The use of α-L-rhamnosidase improve the bioavbility of citrus fruit and citrus fruit wastes beneficial for human health as direct drugs or as nutritional supplements has been reported (Manazanaes *et al.,* 2007, Puri *et al.,* 2005). In this project work, α-L-rhamnosidase secreted by *A. terreus* which is isolated from decayed orange fruit peel wastes, identified by Institute of Microbial Technology and Gene bank Chandigarh as MTCC no. 3374. α-L-rhamnosidase secreted by *A. terreus* using citrus fruit peel as inducer in liquid culture growth medium. This α-L-rhamnosidase activity was successfully used in hydrolytic reaction of orange peel naringin to produce bioavailable, pharmaceutical compounds prunin and L- rhamnose. The hydrolytic reaction is shown in scheme 1.



Scheme 1. Enzymatic De- rhamnosylation of orange peel naringin in to prunin

MATERIALS AND METHODS

Chemicals p-nitrophenyl-α-L-rhamnopyranoside, naringin, L-rhamnose, naringenin, and CM cellulose were purchased from Sigma Chemical Company, St. Louis (USA). Silica powder, $MnSO_4$, NaCl, sodium acetate were from Merck Ltd. Mumbai, India and acetic acid, tartaric acids, citric acids succinic acids and other chemicals from s.d. fine chem. Ltd. Mumbai, India and were used without further purifications. The chemicals including the protein molecular weight markers used in the SDS-PAGE and native-PAGE analysis were procured from Bangalore GENEI Pvt. Limited Bangalore (India). Orange was purchased from local market, washed with distilled water thoroughly before use.

Fungal Strain: The indigenous fungal strain *Aspergillus terreus MTCC 3374* was isolated from decayed orange peel and purified in the laboratory, identified and deposited at MTCC Center and Gene Bank, Institute of Microbial Technology, Chandigarh, India with MTCC No. 3374. The fungal strain was maintained in the laboratory on the Czapek's agar slant at 25°C.The culture condition for optimum production of α-L-rhamnosidase has already been reported (Yadav *et al.,* 2012).

Enzyme assay: The activity of α-L-rhamnosidase was assayed using p- nitrophenyl-α- L- rhamnopyranoside as the substrate and monitoring the liberation of p- nitrophenol spectrphotometrically at λ= 400 nm using molar extinction coefficient value of 21.44 $mM^{-1}cm^{-1}$ by using reported method (Romero *et al.,* 1985). The assay solution 2.5 ml contained 0.3mM p-nitrophenyl-α- L- rhamnopyranoside in 0.2M sodium acetate/ acetic acid buffer pH 4.5 at 57°C, 100 µL of the culture filtrate was added and aliquots ware withdrawn at regular intervals of 2 min. All Spectrophotometric measurements were made with UV/Vis spectrophotometer Hitachi (Japan) model U-2000 which was connected to electronic temperature control unit.

The least count of the absorbance measurements was 0.001 absorbance unit. One enzyme unit is the amount of the enzyme which librates one µmole of p- nitro phenol/min at 57°C under the stander assay conditions specified above. The steady state velocity measurements were reproducible within 5% standard deviation.

Purification of enzyme: For the purification of α-L-rhamnosidase, the fungal strain was grown in four sterilized 250 ml culture flasks each containing 100ml liquid culture medium amended with 3 g of corn cob. On third day of inoculation of the fungal spores when α-L-rhamnosidase activity reached maximum value, the cultures were pooled, mycelia were removed by filtration through four layer of cheese cloth and culture filtrate 200ml with 0.20 IU/ml activity was centrifuged using Sigma refrigerated centrifuge model 3K30 at 8,000 rpm for 20 minutes at 4 °C to remove the particles. The desired enzyme was concentrated by ultra filtration using amicon concentration cell model 8200 using PM 10 membranes. The concentrated enzyme was centrifuged and dialyzed against 0.01 M sodium acetate/ acetic acid buffer of pH 5.0 using 1: 1000 excess of the buffer with three changes

in the 24 hours. The appropriate condition for the binding of α-L-rhamnosidase on DEAE was determined experimentally (Pharmecia FCAB, Uppsala, Sweden 1983). Dialyzed enzyme was loaded on DEAE column of size 1.00 × 22 cm equilibrated with 0.01 M sodium acetate/ acetic acid buffer of pH 5.0. The absorbed enzyme was washed with 50 ml the same buffer and was eluted applying the linear NaCl gradient of 0-1 M in the same buffer (50 ml of the buffer + 50 ml buffer containing 1 M NaCl). The 4.0 ml fractions were collected and were analyzed for the activity of α-L-rhamnosidase ((Romero *et al.,* 1985) and for protein concentration using Lowry's method. The α-L-rhamnosidase active fractions were combined and concentrated by putting in dialysis bag which was kept in solid powdered sucrose. The purified, concentrated enzyme sample 2.0 ml was stored in the refrigerator and was used whenever required. The enzyme did not lose its activity for two months under these conditions.

SDS- *polyacrylamide* and Native- *polyacrylamide* Gel electrophoresis: The homogeneity of the enzyme preparation was checked by SDS-PAGE analysis and molecular mass was determined using the reported method (Weber and Osborn 1969). The seperating gel was 12% acrylamide in 0.375 M Tris-HCl buffer of pH 8.8 and stacking gel was 5% acrylamide in 0. 5 M Tris-HCl buffer of pH 6.8. Proteins were visualized by staining with Coomassie Brilliant Blue R-250. The molecular weight markers used were phosphorylase B (97.4 kDa), bovine serum albumin (66.0 k Da), ovalbumin (43.0 kDa), carbonic anhydrase (29.0) and soyabean trypsin inhibitor (20.1 kDa). The gel was run at the constant current of 20 mA using electro gel 50 equipment of Tecnosource, Mumbai, India. The molecular mass of the purified enzyme was determined by plotting the log of the molecular masses of the protein molecular mass markers versus relative distances travelled by the protein molecular mass markers on the SDS polyacrylamide gel. The log of the molecular mass of the purified enzyme was read corresponding to the relative mobility of the purified enzyme and the molecular mass was calculated from the corresponding value of the log of molecular mass of the enzyme. The native polyacrylamide gel electrophoresis was done using the reagent kit supplied by Bangalore GENEI Pvt. Limited Bangalore (India). The resolving gel was 10% acrylamide in1.5 M Tris – HCl buffer of pH 8.8 and the stacking gel was 5% acrylamide in 0.5 M Tris – HCl buffer of pH 6.8. The reference protein used was bovine serum albumin (66.0kDa). The proteins were located by Coomassie Brilliant Blue R-250.

Circular Dicromism analysis of enzyme: CD is a standard tool for studying the secondary and tertiary structure of proteins; this technique was employed in order to identify the specific nature of the conformational alterations accompanying the ultraviolet-induced inactivation of α-L-rhamnosidase (Kelly and Nicholas 2000). Since enzyme concentrations were kept constant and absorbance varied inversely with wave length. Spectra were divided into three overlapping regions for examination. Analysis were done in saif department, IIT, Indore, M.P. India.

Steady - state kinetics of the enzyme: The value of K_m and Vmax of the purified enzyme for the substrate p-nitrophenyl-α-L-rhamnopyranoside ware determined by measuring the steady state velocity of the enzyme catalyzed reaction at different concentrations of p-nitrophenyl-α-L-rhamnopyranoside (0.1 mM to 3.0 mM) using the reported method (Romero *et al.,* 1985). The K_m and V_{max} value were calculated by linear regression analysis of the data points of the double reciprocal plot. The pH optimum of the purified enzyme was determined by using p-nitrophenyl-α-L-rhamnopyranoside as the substrate and measuring the steady state velocity of the enzyme catalyzed reaction in solutions of varying pH in the range 3.0 to 7.0. The buffers used were 0.2 M sodium acetate/acetic acid. The steady state velocity was plotted against pH of the reaction solutions and pH optimum was calculated from the graph. The pH stability of the enzyme was determined by incubating the enzyme in the buffer of different pHs for 24 hrs at 25°C. The residual activity were assayed and plotted vs. pHs to which the enzyme was exposed for 24 hrs. The temperature optimum was determined by measuring the steady state velocity of the enzyme catalyzed reaction in solutions of varying temperatures (20-80°C) using p-nitrophenyl- α-L-rhamnopyranoside as the substrate. The steady state velocity of the enzyme catalyzed reaction was plotted against the temperature of the reaction solution and temperature optimum was calculated from the graph. The thermal stability was determine by incubating the aliquots of the enzyme at different temperatures (viz. 20°,30°,35°,40°,45°,50°,55°,60°,65°,70°75°,80°,and 85°C) and assaying the residual activity at the interval of 20 minutes for two hrs. The residual activity was plotted against the time for which the enzyme was exposed at that temperature. The energy of deactivation Ea, for thermal denaturation of the enzyme was determined by calculating the rate constants from the half life time of the denaturation curves using the equation $k = 0.693 / t_{1/2}$, and then using the Arrhenius plot between log k and 1/T using the equation log k = log A- Ea/2.303 RT, where A is the frequency factor, Ea is energy of activation, R is molar gas constant and T is temperature in Kelvin. The slope of the straight line gave the value of Ea / 2.303R from which Ea, the energy of activation, was determined.

Effects of a few natural rhamnoglycosides, aglycones and carbohydrates on the activity of the enzyme: The effects of naringin, rutin, hesperidin, naringenin, quercetin, rhamnose and glucose on the activity of the enzyme were determined in the presence of different concentration of these compounds in the range 0-100mM of rhamnoglycosides, aglycones and 0-500 mM of carbohydrates, keeping the concentration of p-nitrophenyl-α-L-rhamnopyranoside fixed at 1.5 mM. The percent residual activity was plotted against the concentration of above compounds.

Effect of metal ions and organic compounds on the activity of α-L-rhamnosidase: The effect of several metal ions viz Mn^{2+}, Cu^{2+}, Ca^{2+}, K^{+}, Zn^{2++}, Na^{+},Fe^{2+}, Mg^{2+} on the activity of the enzyme were studied by adding 5-100 mM of the ions in the reaction solution and determining the activity. The effects of various concentrations of alcohols viz. ethanol, n-propanol and butanol ranging from 5-100 mM on the activity of the enzyme were studied, keeping the substrate concentration (1.5mM) constant and determining the activity of the enzyme. The various concentrations of acids and alcohol viz. citric acid, tartaric acids (5 - 100 mM) were used to determine their effects on the α-L-rhamnosidase activity.

Enzymatic hydrolysis of orange peel naringin using purified enzyme: In 3.0 ml solution of 1mM naringin in 0.5 M sodium phosphate buffer pH 6.5 at 57°C, 0.75IU of the purified enzyme was added and UV/Vis spectrum was recorded in the range 200-800 nm at regular intervals of 15 minutes. The reaction solution was left over night and the

release of L- rhamnose was detected by thin layer chromatography using silica gel on glass plates. The mobile phase used was chloroform-methanol mixture 70:30 (v/v). The release of prunin was also detected by thin layer chromatography using silica gel on glass plates and using butanol: acetic acid: water (40:11:29) (v/v) as mobile phase. The detection was made in iodine chamber. Another experiment was performed using orange peel shocked in ethanol at overnight. pH of ethanolic solution of orange peel was maintained at pH 4.5 by sodium acetate/ acetic acid buffer and treated by α-L-rhamnosidase (0.82 IU) at 50ºC. The reaction solution was left overnight and the release of prunin was detected by thin-layer chromatography process describe above. The release of L-rhamnose and prunin were detected by above process using thin-layer chromatography.

HPLC-MS analysis: Mass spectral data provide structural information on flavonoids and are used to determine molecular masses and to establish the distribution of substituent. HPLC coupled with diode array and mass spectrometric, Model MS rout JMS-600H, Jeol Japan, was used to identify flavonoids in a reaction mixture. All the analysis was done in CDRI, Lucknow, U.P. India.

RESULTS AND DISCUSSION

Purification of α-L-rhamnosidase: The purification procedure of the α-L-rhamnosidase from the culture filtrates of *A. terreus* MTCC-3374 using orange peel as inducer. The elution profile of the enzyme from the CM-Cellulose column shows about 18 fold purification of the enzyme with 320% recovery of the activity. The enzyme was purifying using a simple procedure as compared to the reported procedures [9].

The result of SDS-PAGE analysis is shown in Fig.1 (a). The presence of a single protein band in lane 2 showed that the enzyme was pure. The molecular mass calculated from the SDS-PAGE analysis was 79.0 kDa. The result of native – PAGE analysis of the purified enzyme is shown in Fig.2 (b) in which the bovine serum albumin (66 kDa) has been loaded in lane 1 and purified enzyme was loaded in lane 2. The native PAGE analysis shows that the purified enzyme gave a single protein band which confirmed that the enzyme was pure.

CD spectrum of enzyme: In enzyme, proteins possess a number of chromophores which can give rise to CD signals. The far UV region (240-190 nm) CD spectra associated with various types of secondary structural features such as α-helix, antiparallel β-sheet and triple helix (Puri and Kaira 2005, Yanai and Sato 2000). Studies of far UV, CD of α-L-rhamnosidase of *A. terreus* MTCC- 3374 are shown in Figure 3. The CD spectra can be used to assess quantitatively the overall secondary structure found in enzyme.

Steady state kinetic of the enzyme: The Michealis constant (K_m) and maximum reaction velocity (V_{max}) were determined for the purified enzyme using p-nitro phenyl - α-L-rhamnosidase as the substrate by Lineweaver-Burk plot Figure not shown here. The K_m was 1.50 mM and V_{max} value was found to be 34.5 µmole/min/mg, respectively, at optimum reaction conditions. The K_m values for α-L-rhamnosidases purified from *Aspergillus niger* 1344, from *Bacteroides* JY-6, from *Pseudomonas paucimobilis* FP 2001, from *Aspergillus aculeatus* RhaA and RhaB, *Acrostalagmus luteo albus* and *Aspergillus flavus* MTCC 9606 have been reported to be 1.9, 0.29, 1.18 0.06, 1.52 and 0.30, 2.80mM, 3.38 and 1.89, respectively (Yu *et al.,* 2002,Park *et al.,* 2005, *et al.,* 1999, Yadav *et al.,* 2000, Busto *et al.,* 2007, Manzanres *et al.,* 1997).

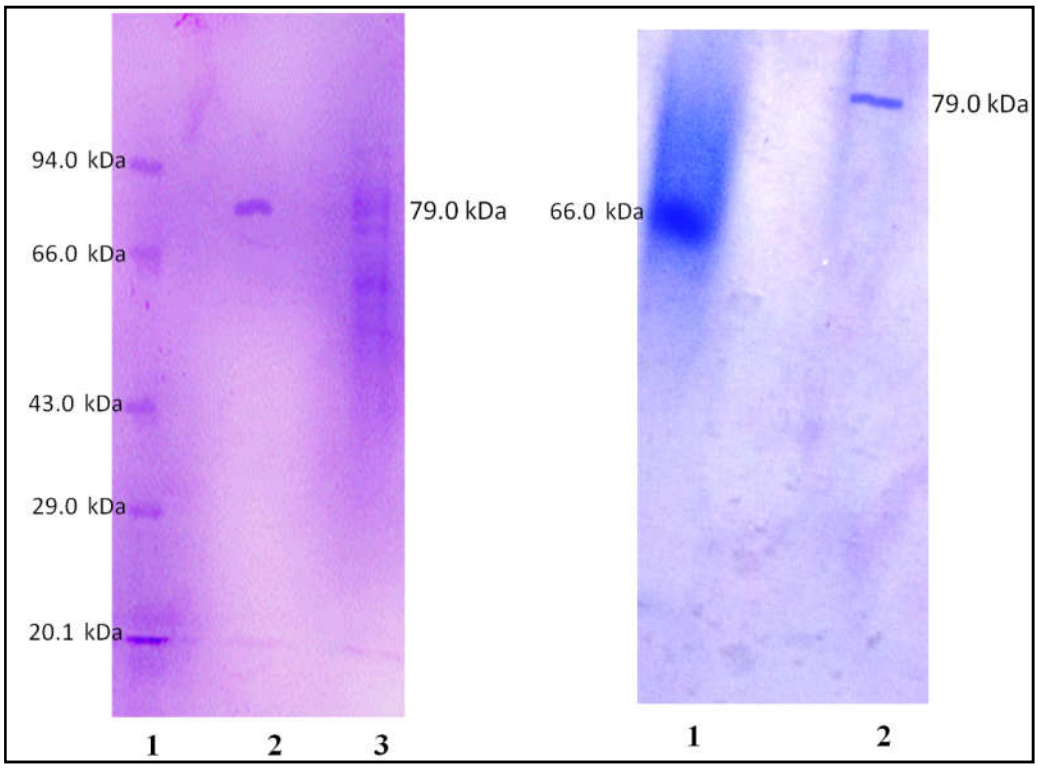


Fig.1. Results of (a) SDS PAGE and (b) Native-PAGE analysis of purified enzyme:
(a) Lane-1 (purified enzyme 0.60 µg) and lane-2 (Molecular Weight Markers).
(b) Lane-1 (Purified enzyme 1.2 µg) and Lane-2 Molecular Weight Marker: Bovine serum albumin (66.0 kDa).

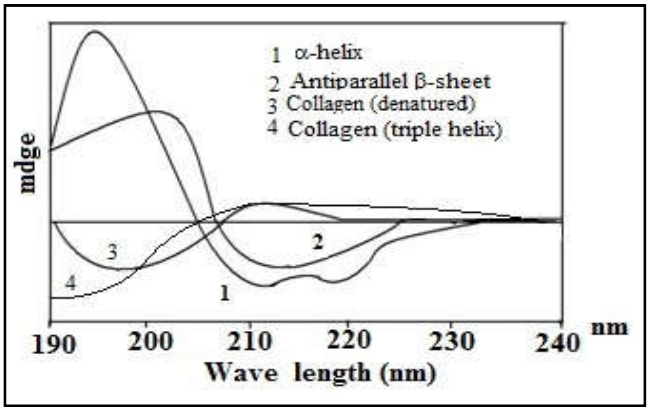


Fig.2. CD spectra of α-L-rhamnosidase

Thus the purified α-L-rhamnosidase has intermediate affinity for p-nitrophenyl-α- L-rhamnopyranoside as compared to reported α-L-rhamnosidases. The calculated k_{cat} value is 31.03 s^{-1} giving k_{cat} / K_m 2.07 × 10^4 $M^{-1}s^{-1}$.

Effect of pH and temperature: The results of studies on the variation of the activity of the purified enzyme with the variation of the pH of the reaction solution are shown to the pH optima of the purified enzyme was found to be 3.5 using p-nitrophenyl- α-L-rhamnopyranoside as the substrate. Most of the α-L-rhamnosidases reported so far have pH optima in the acidic (Yu *et al.,* 2002, Yadav *et al.,* 2000, Vila-Real *et al.,* 2011), basic (Busto *et al.,* 2007, Manzanares *et al.,* 1997,Mueller *et al.,* 2018) and neutral (You *et al.,* 2010, Daniels *et al.,* 1990) pH range. The purified α-L-rhamnosidase has pH optimum in acidic pH range which is suitable for the debittering of orange juice and hydrolyze orange peel naringin. The results of the studied on pH stability of the purified α-L-rhamnosidase show that the enzyme retained most of its activity in buffers of the pHs 2.0-5.0 if left for 24 hrs. The temperature optimum of the enzyme is 55ºC which is in the range 40-80ºC reported for other α-L-rhamnosidases in the literature (Monika *et al.,* 2018, Yadav *et al.,* 2000, Vila-Real *et al.,* 2011). The activation energy for the thermal denaturation of the pure enzyme was calculated by Arrhenius plot was 20.58 kJ/mol/°K. On the basis of the above studies, the purified enzyme can be stored at pH 5.0 in the refrigerator.

Studies for the suitability of the enzyme for debittering: One of the applications of α-L-rhamnosidase is in the debittering of citrus fruit juices (Yadav *et al.,* 2010). Citrus fruit juices contain a number of metal ions, carbohydrates, flavonoids and carboxylic acids. In order to check the suitability of the purified α-L-rhamnosidase for debittering application (Yadav *et al.,* 2017), it is essential to know how the activity of the enzyme is influenced by the flavonoids, carbohydrates and metal ions present in the citrus fruit juices. The results of the effects of flavonoids on the activity of enzyme are shown in Fig. 3(a). The results of the effects of aglycons of the favonoids shown in Fig. 3 (b) and the effect of carbohydrates present in citrus fruit juice on the activity of enzyme are shown in Fig. 3 (c). In all the cases, the activity of enzyme was inhibited by all the components present in citrus fruit peel/juice. The order of inhibition in decreasing order was rutin > hesperidin > quercetin > naringin > naringenin > glucose > rhamnose. Even 5mM concentrations of Mn^{2+},Ca^{2+} and K^+ do not inhibit the activity of the enzyme whereas $Cu2^+$,Na^+, Zn^{2+}, Mg $^{2+}$and Fe^{2+} inhibit the enzyme activity up to 50-80 percent at the same concentrations.

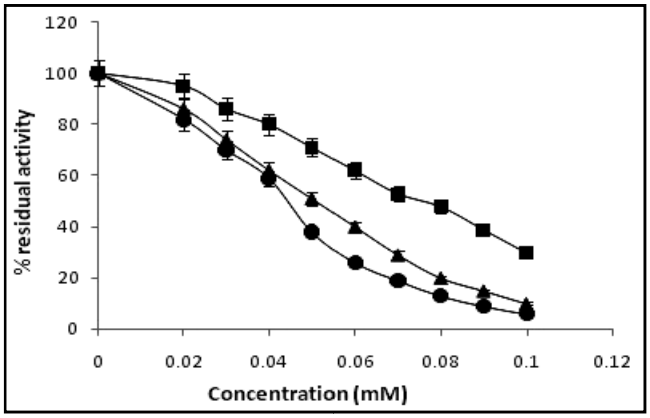


Fig. 3(a). Effect of Naringin (■) , Rutin (▲), Hesperidin (●).

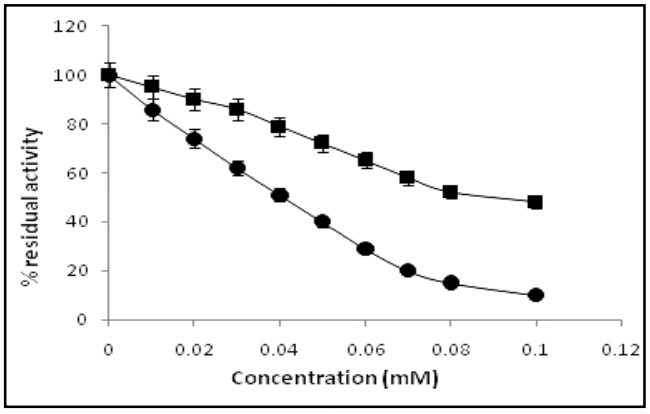


Fig. 3(b). Effect of Naringenin (■), Quercetin (●)

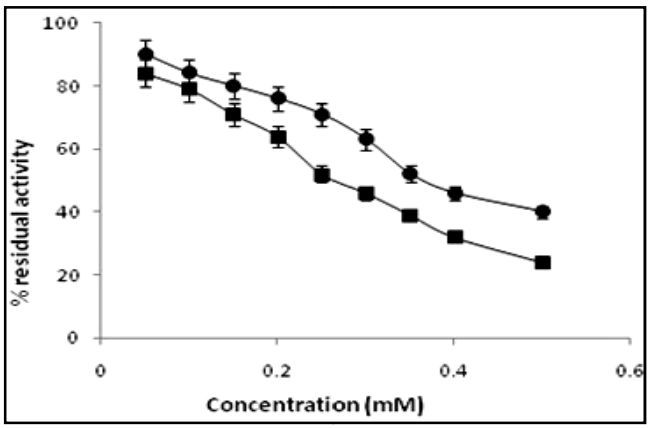


Fig. 3(c). Effect of Carbohydrates i.e rhamnose (●), Glucose (■)

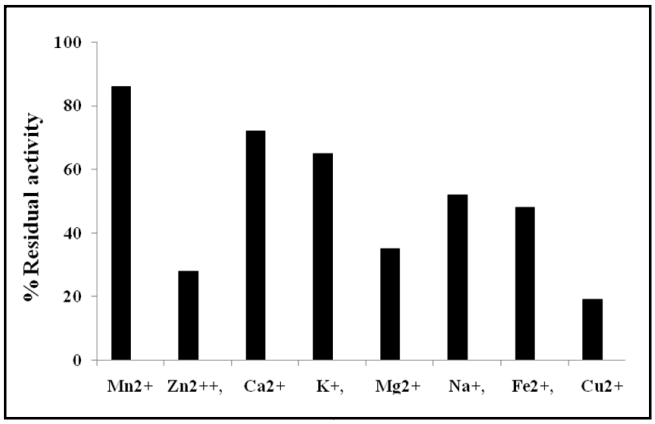
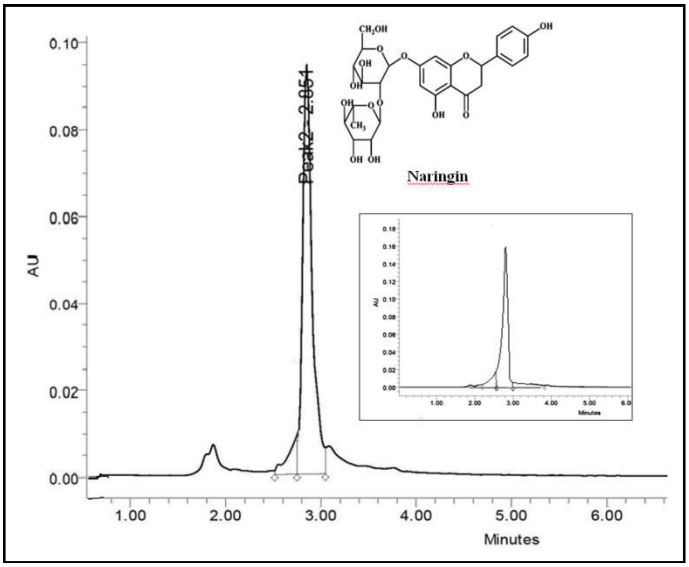
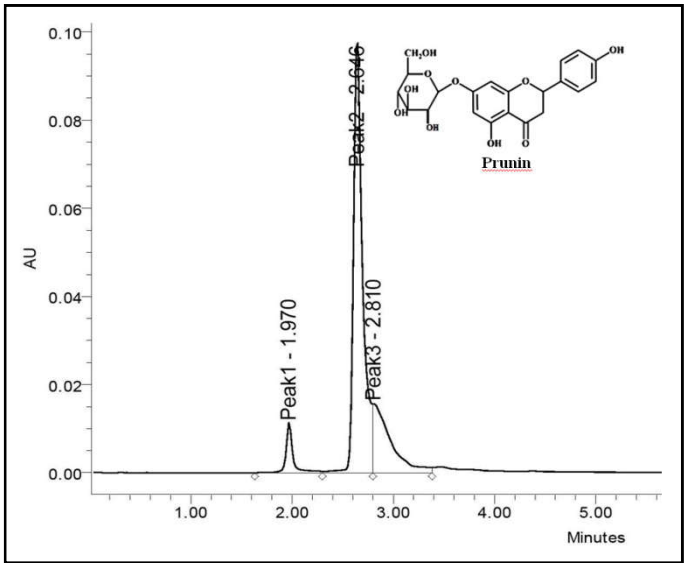


Fig. 3(d). Effects of metal ions

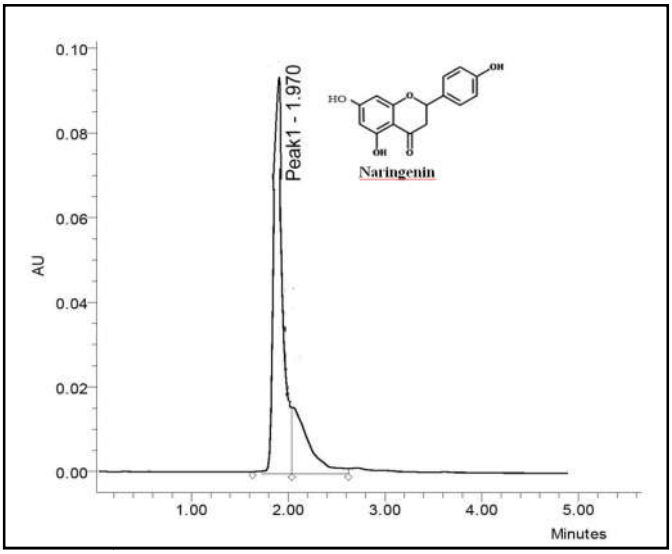
Fig.3. The results of inhibition study on the activity of the enzyme



(a)



(b)



(c)

Fig.4. HPLC Chromatogram of (a) orange fruit peel naringin, In inset stander sample of naringin (b) derhamnosylated product of naringin, (c) Stander sample of naringenin.

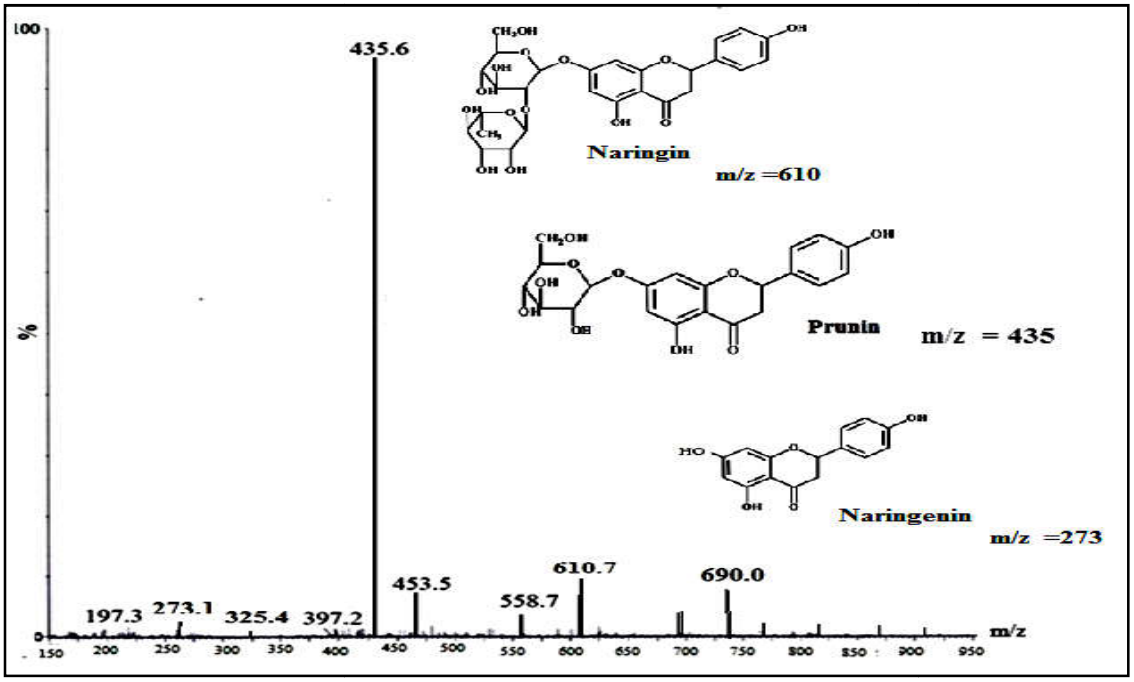


Fig. 5. Results of Mass Spectra of derhamnosylated naringin (prunin) purified by preparative TLC.

Thus the purified enzyme seem to useful for debittering of citrus fruit juices and hydrolyzed the orange peel naringin.

Derhamnosylation of naringin from orange peel: Another application of α-L-rhamnosidase is in derhamnosylation of natural glycosides to produce L- rhamnose and pharmaceutically important derhamnosylated products. With this objective in mind, enzymatic hydrolysis of naringin to produce prunin by the enzyme was tested by TLC. The R_f (0.63) value of enzymatically derhamnosylated product is different from the R_f value (0.39) of the starting compound naringin and also from the R_f value (0.69) of deglycosylated product of naringin i.e. naringenin, it is inferred as prunin.

Analysis of the reaction products by HPLC–MS: For analysis of HPLC-MS derhamnosylated product was purified by preparative TLC technique. HPLC–MS analysis of hydrolyzed naringin after 2h α-L-rhamnosidase reaction shows that conversion of 48% of naringin into prunin (Fig. 4 a,b,c). Derhamnosylated product of naringin was detected by HPLC–MS in negative ion mode, as a peak at 2.64 min (m/z 435) which corresponded to its de-protonated ion [M – H], and confirmed by comparison of the retention time and MS/MS fragments of a standard of naringin and its aglycons naringenin (Fig. 5). From this study it is shown that α–L- rhamnosidase from *A. terreus* MTCC-3374 convert orange peel naringin in to the more bioavailable prunin (naringenin -glucoside) by de-rhamnosylation.

Conclusion

In conclusion, this communication reports the purification of an α-L-rhamnosidase from the culture filtrate of a new fungal strain *A. terreus MTCC-3374*, isolated from decayed orange fruit peel, using a simpler procedure compared to the procedures reported for the purifications of the other fungal α-L-rhamnosidases. The enzyme is active in acidic pH range and can be used for the preparation of L-rhamnose and prunin from ethanolic solution of orange fruit peel. The enzyme is specific for the conversion of naringin to prunin which is a pharmaceutically important bioavailable, rare compound of medicinal and food values.

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