Research Article

Characterization of antioxidants from two varieties of *Azadirachta indica* and *Melia azedarach*

S. Anbuselvi*, L. Jeyanthi Rebecca

ABSTRACT

Objective: Plant enzyme peroxidase was extracted from different varieties of neem and its purification by gel filtration. Medicinal plants are premier adapt for helping the body and mind to adopt and cope with a wide range of physical, emotional, chemical, and infectious stresses and restore physiological and psychological functions to a normal healthy state. The isoenzyme, peroxidase stands responsible for their health beneficial antioxidant capacity which, in turn, contributes toward their medicinal properties. **Results and Discussion:** *Azadirachta indica* and *Melia azedarach* varieties of neem were studied for peroxidase analysis. The optimum pH and temperature of peroxidase were found to be 6.5 and 40°C. This was studied to understand the variation between single species at protein level. Protein profiling was done in both native PAGE and SDS.

KEY WORDS: Neem, Peroxidase, Protein purification, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

INTRODUCTION

Peroxidases are widely distributed in nature and are produced by a wide variety of plant species, the chief commercial source being horseradish (*Armoracia rusticana*) and soybean (*Glycine max*).[1] Peroxidases are heme-containing enzymes that oxidize a wide variety of organic and inorganic substrates by reducing hydrogen peroxide and peroxides.[2] Peroxidase is a heat stable enzyme, preferring the preparation of enzyme-conjugated antibodies and other sensitive analytical techniques.[3] They play critical roles in physiological functions such as cell metabolism, plant resistance, and auxin catabolism.[4-6] This also commonly used in pharmaceutical industry, especially glucose estimation drugs.

Two varieties of neem, namely *Azadirachta indica* and *Melia azedarach,* were considered for the work. The chemical composition of neem is highly complex and contains many nutrients and biologically active compounds. The chemical compounds that have been identified as volatile oil, terpenoids, eugenol, thymol, estragole, and ursolic acid.[7] Neem is used as an expectorant, bronchitis, ringworm, and other cutaneous diseases, stomachic, gastric disorders, and earache of children and also reduces the toxicity. It also enhances general health and well-being, having positive overall effects on the body and mind.[8]

Neem has antibacterial properties that help in fighting against skin infections such as acne, psoriasis, scabies, and eczema. Neem extracts also help in treating diabetes, AIDS, cancer, heart disease, herpes, allergies, ulcers, hepatitis, and several other diseases. Neem oil, leaves, and neem extracts are used to manufacture health and beauty care products. Neem leaf tablets to increase immunity, insect repellents, nematicide, contraceptives, pet care products, and various disease control.[9,10]

The main objective of this work was to isolate peroxidase from two varieties of commonly found medicinal plants and its partial purification by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The isoenzyme patterns were identified by native PAGE. The affinity chromatography was used for complete purification and its properties.

MATERIALS AND METHODS

Tulsi varieties were identified by botanists Rama and Krishna neem varieties (*A. indica* and *M. azedarach*

Access this article online

Website: jprsolutions.info | ISSN: 0975-7619

Department of Industrial Biotechnology, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India

***Corresponding author:** S. Anbuselvi, Department of Industrial Biotechnology, Bharath Institute of Higher Education and Research, Chennai - 600 073, Tamil Nadu, India. E-mail: anbuselvichennai@yahoo.com

Received on: 14-01-2018; Revised on: 22-02-2019; Accepted on: 19-03-2019

were collected). The leaves of all the varieties were used for the estimation and isolation of peroxidase. 500 mg of all the two leaf samples were weighed and grinded with the addition of 1 ml of phosphate buffer (pH7). This was then centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant was passed through filter paper.[1,11] It was heated in a water bath at 65°C for 3 min to inactivate catalase in the extract and cooled promptly by placing in ice bucket for 10 min.

Purification of Peroxidase

The enzyme extract was subjected to ammonium sulfate precipitation by the method of Evans[12] and the precipitate was subjected to gel filtration in the form of desalting column.

Desalting Column

Desalting columns are pre-packed, ready to use columns for group separation between high- and low-molecular-weight substances. The desalting columns are prepared by packing size exclusion matrix. The matrix is beds of cross-linked dextran with epichlorohydrin. The fractionation range for globular proteins is between 1000 and 5000 Da.

Assay of Peroxidase Activity

Assay of peroxidase activity and substrate specificity was performed according in the method by Koksal and Gulcin (2008). 50 µl of enzyme solution was added to the mixture of 1 ml 260 mM hydrogen peroxide and 50 µl of 20 Mm guaiacol and the mixture was adjusted to 2 ml by adding 0.1 Mm sodium phosphate buffer (pH 6.0). The absorbance of colored complex was read at 470 nm after 3 min reaction interval (Ambreen *et al*., 2000). Protein content of the enzyme extract at all steps was measured by Bradford method.[13]

SDS-PAGE and Native PAGE

SDS-PAGE was performed according to the Laemmli's system[14] using 12.5% polyacrylamide gel. For native PAGE, the sample extract is electrophoresed in starch or polyacrylamide buffered (non-denaturating) stack gel at a low temp. (4°C–8°C). Each lane should be loaded with equal amount of proteins after normalizing the proteins content in exact in as small volume as possible (25–50 µl). Later electrophoresis, the gel is incubated in a solution containing all the necessary components for enzyme reaction. The colored reaction product stains the gel where the enzymes were located. The separation of native (non-denatured) proteins in this method was based on both the charge and size of the proteins. Since the native proteins are large and exist in quaternary structure, a gel of low percentage of acrylamide was used.[15]

Effect of Ph and Temperature on Peroxidase Activity

The optimum pH was determined by assaying enzyme activity at different pH levels. The assay was performed by taking buffers at different pH such as 0.1 M sodium acetate buffer (pH 3.0–5.0) and 0.1 M sodium phosphate buffer (pH 5.5–6.5). The optimum temperature of peroxidase was determined by assaying the enzyme activity with different range of temperature from 20°C to 60°C. The thermostability of peroxidase was measured by incubating the enzyme at 60°C and the activity was measured every 10 min. Peroxidase activity was assayed using guaiacol substrate.

RESULTS AND DISCUSSION

After gel filtration of two varieties of neem the protein content of the crude and the purified samples were estimated by Bradford's methodology using standard bovine serum albumin (BSA). Table 1 showed the amount of protein present in the different samples, and it was seen that the crude extract was found to be higher in crude form than the purified sample.

Protein content of *A. indica* was found to be higher (15.86 mg/mL) for the crude extract and

Table 1: Partial purification of peroxidase of *Azadirachta indica*

Purification step	Total volume (mL)	Protein content (mg/mL)	Enzyme activity (U/mL)	Specific activity (U/mg)	Fold purification
Crude extract	500	15.86	18.68	2.58	1
Ammonium sulfate precipitation	280	8.35	13.56	5.63	2.78
Gel filtered extract	25	4.67	10.9	12.34	8.99

Table 2: Partial purification of peroxidase of *Melia azedarach*

Purification step	Total volume (mL)	Protein content (mg/mL)	Enzyme activity (U/mL)	Specific activity (U/mg)	Fold purification
Crude extract	500	8.67	12.5	2.14	1.2
Ammonium sulfate precipitation	235	5.78	9.65	5.76	2.9
Gel filtered extract	20	0.78	6.34	14.56	7.86

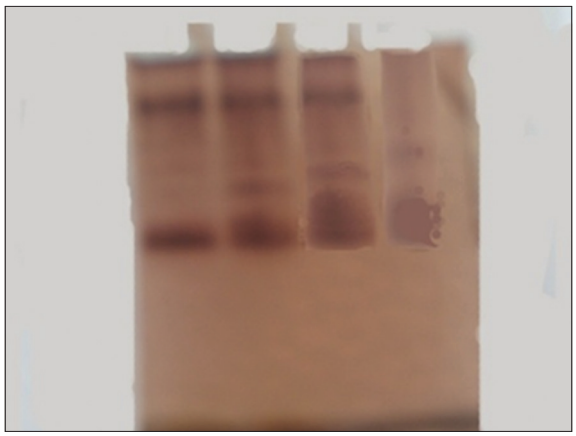

Figure 1: Native polyacrylamide gel electrophoresis of peroxidase from two varieties of tulsi and neem

4.67 mg for the purified protein when compared with *M. azedarach* [Tables 1 and 2]. To purify the enzyme, the crude extract was subjected to ammonium sulfate to remove unwanted proteins. This is the most commonly used reagent for salting out of proteins due to its high solubility permits the achievements of the solution with high ionic strength.[16,17] The degree of purification of desalted enzyme was increased by applying into gel filtration. The maximum specific activity of the peroxidase extracted from *M. azedarach* was 14.56 U/mg. The results of our findings which exhibited maximum fold of purification (8.99) and maximum enzyme activity were also observed in *A. indica* [Tables 1 and 2].

Gel filtration which proved efficient as a fraction showed maximum (8.99) fold purification in *A. indica* and high specific activity (14.56 U/mg) was found in *M. azedarach* among four different samples. Zia (2002) found 18.644-fold purification after applying horseradish peroxidase to gel filtration chromatography. The protein and isoenzyme pattern of peroxidase were confirmed by SDS-PAGE (not shown) and native PAGE [Figure 1], *A. indic*a and *M. azedarach.*

The zymogram [Figure 1] obtained showed the migration of the enzyme peroxidase to different levels under the electric field. The protein molecules migrate to different levels in accordance of their charge and molecular weight, thus from the zymogram, it was seen that the peroxidase isolated from *A. indica* was having a higher molecular weight than *M. azedarach.* It was known that pH and temperature were the key factor for enzyme activity and it changes ionization state of protein and substrate.[18] The optimum pH was found to be around d 6.5 in and neem varieties. The peroxidase shows optimum activity between pH6 and 8.5.[19] Altunkaya and Gokman suggested that the purified isoenzyme showed pH below 7. The optimum temperature was found to be in the range of 40°C–45°C.[20-22]

CONCLUSION

Peroxidase was isolated and estimated from the two neem varieties *A. Indica* and *M. azedarach.* From this study, it was found that the protein content and its enzyme activity were different for varieties within the same species. This study was helpful in understanding the varietal difference within the same species. Local availability of these plants and reasonably high specific activity of the enzymes isolated from these medicinal plants makes it a better choice for the production of peroxidase for its use as antioxidant.

REFERENCES

1. Ambreen S, Rehman K, Zia MA, Habib F. Kinetic studies and partial purification of peroxidase in soybean. Pak J Agric Sci 2000;37:119-22.
2. Kvaratshkhelia M, Wrinkel C, Thomele RN. Production of peroxidase from medicinal plants. Plant Physiol 1997;114: 1237-45.
3. Barnes CS, Upadrashta B, Pacheco F, Porton J. Enhanced sensitivity of immunoblouing with POD conjugated antibodies using an adsorbed substrate method. J Chem 1993;61:281-8.
4. Boka K, Orban N. Dynamics and localization of in plant adaptation to temperature. Plant Signal Behav 2007;2:498-500.
5. Hiraga S, Sasaki K, Ito H, Ohashi Y, Matsui H. A large family of class III plant peroxidases. Plant Cell Physiol 2001;42:462-8.
6. Lagrimini LM, Joly RJ, Dunlap JR, Liu TY. The consequence of peroxidase overexpression in transgenic plants on root growth and development. Plant Mol Biol 1997;33:887-95.
7. Jia J, Wang B, Wu A, Cheng G, Li Z, Dong S, *et al.* A method to construct a third-generation horseradish peroxidase biosensor: Self-assembling gold nanoparticles to three-dimensional sol-gel network. Anal Chem 2002;74:2217-23.
8. Gupta VK, Sharma SK. Plants as natural antioxidants. Natl Prod Radiance 2006;5:326-34.
9. Prajapati ND, Purohit SS, Sharma AK, Kumar T. A Handbook of Organic Farming and Biofertilizers for Sustainable Agriculture. Johdhpur: Agrobios; 2003. p. 321-33.
10. Anbuselvi S, Kumar MS, Padhee K, Kumar S. Isolation and purification of peroxidase from *Pisum sativum* L grown with different orgnic manures. Int J Pharm Technol 2012;4:5011-8.
11. Zia MA. Fermentation biotechnology for production of Industrial Enzymes. M. Phil Biochemistry Thesis, University of Agriculture, Faisalabad: 2002. p. 56-9.
12. Evans JJ. Peroxidases from the extreme dwarf tomato plant. Identification, isolation, and partial purification. Plant Physiol 1968;43:1037-41.
13. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-54.
14. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680-5.
15. Schägger H, von Jagow G. Blue native electrophoresis for isolation of membrane protein complexes in enzymatically active form. Anal Biochem 1991;199:223-31.
16. Voet D, Voet JG, Pratt CW. Fundamentals Biochemistry. Hoboken, New Jersey: John Wiley and. Sons; 1999. p. 687-9.
17. Rehman K, Yaqub M, Sheik MA, Arshad M. Int J Agric Biol 1999;1:170-3.

18. Gawlik-Dziki U, Zlolek U, Swieca M. Characterization of polyphenol oxidase from butter lettuce (*Lactuca sativa* var capitata L.). Food Chem 2008;107:129-35.
19. Onsa GH, Saari N, Selamat J, Baker J. Food Chem 2006;92:365-76.
20. Altunkaya A, Gokmen V. Purification and characterization of polyphenol oxidase, peroxidase and lipoxygenase from freshly cutlettuce (*L. sativa*). Food Technol Biotechnol 2011;49:249-56.
21. Anbuselvi S, Balamurugan T, Senthilkumar P. Purification and characterstics of peroxidase from two varieties of tulsi and neem. Res J Pharm Bio Chem Sci 2013;l4:648-54.
22. Singh N, Gade WN, Singh J. Purification of turnip peroxidase and its kinetic properties. Prep Biochem Biotechnol 2002;32:39-49.

Source of support: Nil; Conflict of interest: None Declared