ARTICLE TYPE

Extraction, and Purification of Peroxidase Enzyme from *Peganum harmala* Seeds

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ABSTRACT

The aim of this study was to extract peroxides enzyme from *Peganum harmala* seeds; peroxides was extracted by using different extraction buffer solutions, then it was purified by three steps of purification includes precipitation with ammonium sulfate in a saturation ratio of 70 %, ion exchange chromatography through DEAE-Cellulose, and gel filtration chromatography throughout Sephadex G-100, and determine the optimum condition for extraction. This was performed by controlling the type and concentration of buffer, pH of the buffer used, and the ratio of extraction. The Sodium acetate buffer with 0.2mM and pH 5.0 was found to be the best buffer for the extraction of peroxidase. By using the extraction ratio for a plant of 1:3 (W/V), the specific activity was 195 U/mg protein. These three purification steps raised the specific activity to 235U/mg protein in the precipitation step with purification fold 2.3 and enzyme recovery 69%; the specific activity was increased to 243U/mg protein in Ion exchange step with purification fold 2.4 and enzyme recovery 23%, also the specific activity doubled after gel filtration step to 447U/mg protein with purification fold 4.4 and enzyme recovery 15%.

Keywords: Extraction, *Peganum harmala*, Peroxides, Purification.

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INTRODUCTION

In the past decades, many in vivo and in vitro studies have shown that medicinal plants had antibacterial activity. World health organization (WHO) estimated that approximately 80% of the people in most of the countries prefer and trust in traditional therapies. The plant extracts its primary medicinal source to treat infectious diseases.[1] Oils or extracts of medicinal plants, which have antimicrobial and anti-inflammatory effects, have been recently used to treat many human infectious diseases.[2] *Peganum harmala L*. is One of the most famous plants used in popular medicine, which belongs to the Zygophyllaceae family.[3] Commonly known as Syrian rue, Wild rue or Harmal is native to arid and semi-arid regions of Asian and Northern African deserts that have spread to parts of the Northern Mexico and the southwestern United States. Pharmacologically active alkaloids 2% - 6% found in *Peganum harmala* seeds, which are mostly β-carbolines like harman, harmine, harmaline, and harmalol. various studies have shown various pharmacological characteristics and biological activities of its seeds such as antimicrobial, antifungal, anti-inflammatory, antidiabetic, anti-cancerous, hypothermic, and hallucinogenic activities. The highest levels of alkaloids usually found in the Seeds and roots, while stem and leaves contain low levels and absent of alkaloids in flowers.[4] it acts as an inhibitor of the enzyme monoamine oxydase (MAO) and myeloperoxidase enzymes.[5] Also, the smoke of harmala seeds is traditionally used as a disinfectant.[6]

The seeds and the whole plant possess medicinal properties (Uighur Drug Standard of the Ministry of Public Health), and Many of reports suggest that the plant can be used to treat ailments such as diabetes, rheumatism, hypertension, jaundice, and asthma. The seeds also possess hallucinogenic and hypothermic properties and are used as a medical remedy, incense, condiment with necrotic, sedative, aphrodisiac, stimulant, and emetic properties. The Seeds are used for the treatment of fever, malaria, hysteria, neuralgia, rheumatism, and syphilis an eye.[7-12] In addition, *P. harmala* is also an anti-parasitic agent. Moreover, the alkaloids identified in *P. harmala* exhibit some pharmacological action, such as antitumor and analgesic effects.[12-14]

Peroxidase (E.C.1.11.1.7) which belongs to the oxidoreductase class of enzyme and generally catalyzes a reaction between H_2O_2 as electron acceptor and many kinds of substrates by means of O_2 liberation from H_2O_2.[15] Peroxidases exist in a very wide range of plants, especially at plasmalemma, vacuoles, tonoplast, and inside and outside the cell wall.[16] Also, present in other living organisms such as animals and microorganisms.[17] Peroxidase a widely distributed compound

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in nature, catalyzes the reduction of hydrogen peroxide to water, making it harmless.[18] Peroxidases are found to exist in both membrane-bound and involved in lignin biosynthesis, protective mechanisms, and regulation of plant hormone.[19] Different physiological functions of these enzymes due to the presence of isoenzymes in plant cell organelles. They are include participated in lignifications and plant hormone regulation, suberization, protection toward H_2O_2 and other oxidants, defense properties against pathogenic causes, and play a role in tolerance of drought in a plant. This enzyme used in varied scientific fields involves biotechnology, biochemistry, clinical, and industrial purposes. It used in Immunochemistry and ELASA. Manufacturing of many aromatic complexes, diagnosis, and biosensors for recombination and expression of recombinant protein and protein engineering. Elimination of phenolic complexes from wastewater and peroxides from foods, beverage, and industrial wastes.[20] Because of its several roles, the enzyme is frequently observed as several iso-enzymes in plants, SO The present study was carried out to extracted the peroxidase enzyme from *Peganum harmala* seeds.

MATERIALS AND METHODS

Peroxidase Extraction

Peroxidase was extracted from *Peganum harmala* by mixing 200g of *Peganum harmala* with 0.2 M sodium acetate buffer solution (pH5) in a ratio of 3:1(weight: volume) with continuous stirring for 15 minutes at 4 °C. Mixture was then filtered throughout filter paper, and then the filtrate was centrifuged at 10000 rpm for 15 minutes. Protein concentration, activity, and specific activity of peroxidase were determined.

Protein Concentration

Protein concentration in peroxidase extracts was determined according to Bradford[21] by using Coomassie blue G-250 and Bovine serum albumin standard solution.

Enzyme assay

Peroxidase activity was assayed according to Bernhard and Whitaker.[22] Enzyme activity was defined as the amount of the enzyme oxidizing 1 µmol of glycol in one minute under the experimental conditions.

Purification of enzyme

Three steps of purification for peroxidase extracted from wheat bran include precipitation by ammonium sulfate, Ion exchange chromatography, and gel filtration chromatography.

Ammonium sulfate precipitation

Peroxidase in crude extracts was precipitated by using different saturation ratios of ammonium sulfate ranged between 30% and 80%. Enzyme activity was determined after precipitation with each saturation ratio. The precipitated enzyme was dissolved in distilled water and dialyzed against distilled water for 24 hours at 4°C with three increments of substitutions. Then protein concentration, activity, and specific activity of peroxidase were determined.

Ion exchange chromatography

Ion exchange chromatography was used to purify peroxidase obtained from precipitation step was purified throughout DEAE – cellulose. The washing step was achieved by using phosphate buffer (5mM, pH=7), while the elution step was achieved by using gradient concentrations of sodium chloride (0.1 -1 M). Fractions were collected at a flow rate of 20mL/hour. Absorbance (280nm) and enzyme activity were measured in each fraction.

Gel filtration chromatography

Enzyme solution produced from Ion exchange step was concentrated and purified by gel filtration chromatography throughout Sephadex G-100 and eluted with potassium phosphate buffer solution (0.2M, pH 7), at a flow rate of 20ml/hour. Absorbance (280nm) and enzyme activity were measured in each fraction.

RESULTS AND DISCUSSION

The extraction buffer

Four different pH and two concentrations of sodium acetate were used to determine the best buffer and its concentration for the extraction method of an enzyme from *Peganum harmala* seeds. The results in Figure 1 shown the differences of peroxidase activity according to different buffer concentration and pH, the 0.2Msodium acetate with pH=5 was the best buffer for extraction in which the specific activity of peroxidase was 140 U/mg protein followed by pH=6and 0.2M with specific activity 100 U/mg protein.

The optimum ratio of peroxidase extraction:

Four ratios were chosen (1:2, 1:3, 1:4 and 1:5) (W:V) to determine the best ratio of peroxidase extraction by using sodium acetate buffer 0.2M pH= 5, the specific activity was measured for crude extract when it recorded the highest value in 1:3 ratio, it was 195U/mg protein compared with the other ratios, followed by 1:2 with 160 U/mg protein,. Nidadavolu *et al.,* (2010)[23] were found that the best extraction ratio for peroxidase purified from discarded mushroom beds was 1:1

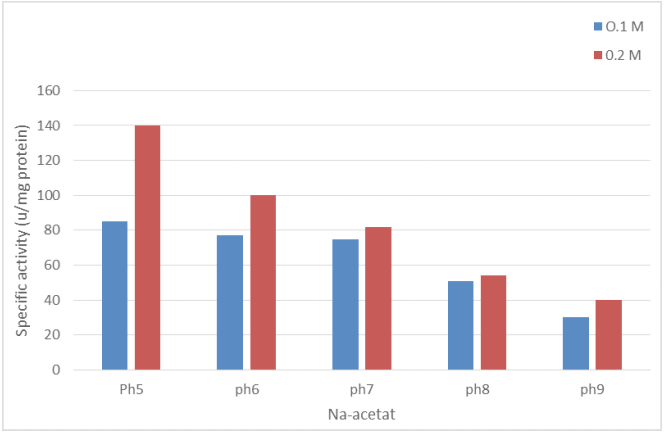

Figure 1: Effect of different buffers on peroxidase extraction from *Peganum harmala* seeds.

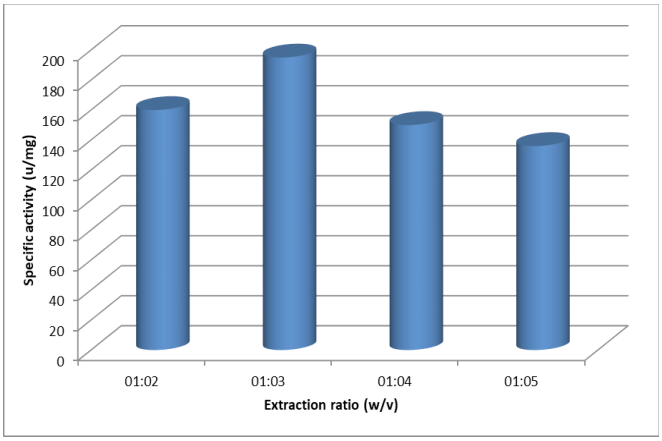

Figure 2: The effect of extraction ratio on peroxidase activity from *Peganum harmala* seeds.

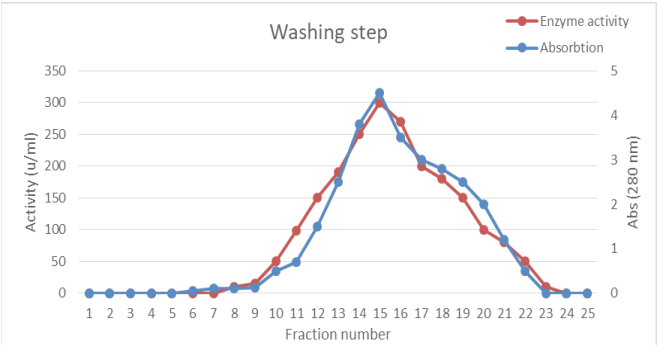

Figure 3: Ion exchange chromatography for purification of peroxidase extracted from *Peganum harmala* seeds using DEAE-Cellulose column (2.5×16) with a flow rate of 20 ml/hour

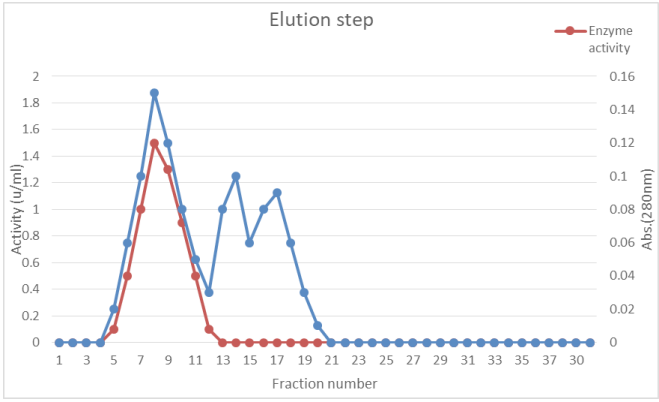

Figure 4: Ion exchange chromatography using DEAE-Cellulose column(2.5×16) cm with gradient salt (0.1-1) M NaCl, flow rate 20mL/hr., and fraction volume 3ml for purification peroxidase from.

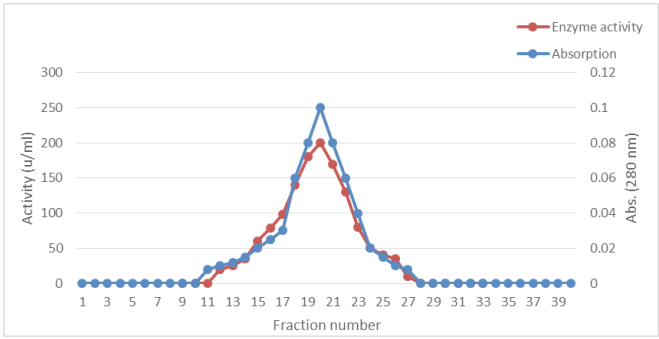

Figure 5: Gel filtration chromatography for purification of peroxidase extracted from *Peganum harmala* seeds using Sephadex G-100 (36×1.5 cm) equilibrated with potassium phosphate buffer solution (0.2M, pH 7) at a flow rate of 20mL/hour.

Table 1: The purification steps of peroxidase extracted from Peganum harmala seeds

Purification step	*Volume (ml)*	*Enzyme activity (U/mL)*	*Protein concentration (mg/mL)*	*Specific activity (U/mg)*	*Total activity (U\mL)*	*Purification fold*	*Yield (%)*
Crude extract	100	400	4	100	40000	1	100
Ammonium sulfate precipitation	30	920	4	235	27600	2.3	69
Ion exchange chromatography	18	512	2.1	243	9216	2.4	23
Gel filtration Chromatography	20	313	0.7	447	6260	4.4	15

and 1:2. While Tsujimura *et al.*,(1994)[24] extracted peroxidase from *Arthromycesramosus* with 1:5 ratio.

Ion exchange chromatography

Dialyzed enzyme produced from ammonium sulfate precipitation step was more purified throughout DEAE-Cellulose ion exchanger. Results illustrated in Figure 3 showed that one peak of proteins that appeared in the washing step represents enzyme activity. This indicates that peroxidase carry positive charge similar to the charge of ion exchange under the experimental conditions. In this step of purification, enzyme-specific activity was 343 U/mg with a purification fold 2.4 and yield reach to 23% as shown in Table 1. While in the elution step illustrated in Figure 4, four protein peaks were eluted by gradient salt concentrations, not represents enzyme activity. In another study, Zia *et al.*,[25] purify peroxidase extracted from apple seeds, and he was found that the specific activity of peroxidase after this step of purification was 7.53 U/mg, with purification fold of 6.82 and 42.89% recovery.

Gel filtration chromatography

Enzyme solution obtained from the ion-exchange chromatography purification step was more purified by Gel filtration chromatography technique throughout Sephadex G-100. One protein peak represents enzyme activity was appeared (Figure 5). Enzyme specific activity was reached 447 U/mg, with a purification fold 4.4 and enzyme yield of 15%, as shown in Table 1.

REFERENCES

1. Folashade O, Omoregie H, Ochogu P. Standardization of herbal medicines-A review. Int J Biodiv Conserv. 2012;4(3):101–12.
2. Goudarzi M, Fazeli M, Azad M, Seyedjavadi SS, Mousavi R. Aloe vera Gel: Effective Therapeutic Agent against MultidrugResistant Pseudomonas aeruginosa Isolates Recovered from Burn Wound Infections. Chemother Res Pract. 2015;2015:639806. doi: 10.1155/2015/639806. [PubMed: 26266047].

3. Nenaah G. Antibacterial and antifungal activities of (beta)-carboline alkaloids of Peganum harmala (L) seeds and their combination effects. Fitoterapia. 2010;81(7):779–82. doi: 10.1016/j.fitote.2010.04.004. [PubMed: 20398742].
4. *(PDF) Distribution, Status, Pharmacological, and Traditional importance of Peganum harmala L*. Available from: https://www.researchgate.net/publication/324923121_Distribution_Status_Pharmacological_and_Traditional_importance_of_Peganum_harmala_L [accessed Dec 12 2018].
5. S Bensalem;J Soubhye; I Aldib; L Bournine *et al*. J Ethnopharmacology. 2014;
6. Mansour M, Soudabe N. The effect of Peganum harmala and Teucrium polium alcoholic extracts on growth of Escherichia coli O157. Jundishapur J Microbiol. 2012;5(3):511–5.
7. A. Astulla, K. Zaima, Y. Matsuno, Y. Hirasawa, W. Ekasari, A. Widyawaruyanti, N.C. Zaini, and H. Morit a Alkaloids from the seeds of Peganum harmala showing antiplasmodial and vasorelaxant activities. J. Nat. Med. 62, 2008, 470–472.
8. H. Berrougui, M. Isabelle, M. Cloutier, M. Hmamouchi, and A. Khalil. Protective effects of Peganum harmala L. extract, harmine and harmaline against human low density lipoprotein oxidation. J.Pharm. Pharmacol, 58, 2006, 967–974.
9. L. Elbahri, and R. Chemli. Peganum harmala L.: a poisonous plant of North Africa. Vet. Human Toxicol. 33, 1991, 276–277.
10. L. Farouk, A. Laroubi, R. Aboufatima, A. Benharref, and A. Chait . Evaluation of the analgesic effect of alkaloid extract of Peganum harmala L.: possible mechanisms involved. J. Ethnopharmacol. 115, 2008, 449–454.
11. H. Monsef, G. Ali, I. Mehrdad, and A. Mohamed. Antinociceptive effects of P. Harmala L. Alkaloids extract on mouse formaline test. J. of pharmacy and pharmaceutical Sci., 7, 2004, 65 – 69.
12. A. R. Shahverdi, S.N.Ostad, S. Khodaee, L. Bitarafan, H. R. Monsef Esfahani,H.Jamalifar, B.Nikavar,M. Mohseni. Antimicrobial and cytotoxicity potential of Peganum harmala smoke. Pharmacogn. Mag, 4, 2008, 236–240.
13. C.H.Wang, J. Liu, and L.M. Zheng."Analysis of harmine and harmaline of Peganum harmala in different parts and different localities." Chinese Pharmaceutical Journal-Beijing, 37, 2002, 211-214.
14. F. Jahaniani, S.A. Ebrahimi, N. Rahbar-Roshandel, M. Mahmoudian. Xanthomicrol is the main cytotoxic component of Dracocephalum kotschyii and a potential anti-cancer agent. Phytochemistry, 66, 2005, 1581- 1592.
15. Zia, M.A.; Kousar, M.; Ahmed, I.; Iqbal, H.M.N. and Abbas, R.Z. (2011). Comparative study of peroxidase purification from apple and orange seeds. *African Journal of Biotechnology*. 10(33), pp. 6300-6303
16. Vitali, A.; Botta, B.; Dellemonache, G.; Zappitelli, S.; Ricciardi, P.; Melino, S.; Petruzzelli, R. and Giardina, B. (1998). Purification and partial characterization of a peroxidase from plant cell cultures of Cassia didymobotrya and biotransformation studies. Biochem., J. 331: 513-519. 4.
17. Chen, E. L.; Chen, Y. A.; Chen, L. M. and Liu, Z. H. Effect of copper on peroxidase activity and lignin content in Raphanus sativus. Plant Physiol. Bioch., 2002; 40: 439-444.
18. A.Steevensz, S.Madur, M.M.Al-Ansari, K.E.Taylor, J.K.Bewtra, N.Biswas; A simple lab-scale extraction of soybean hull peroxidase shows wide variation among cultivars,Industrial Crops and Products, 48, 13-18 (2013).
19. I. S.Hamad, A. A. .Ahmed; Biodegradation of phenolsin wastewater using crude peroxidasesfrom five weed plants,Journal of Chemical and Pharma- ceutical Research, 5, 60-65 (2013).
20. Hamid, M.; Majeed, H.; and Al-Assie, A. H. Purification and Characterization of Peroxidase Extracted from Wheat Bran. World Jornal of Pharmaceutical Research., 2015; Volume 5, Issue 01, 77-86.
21. Bradford, M. M. A rapid and sensitive method for the quantification of microorganism quantities of protein utilizing the principle of protein- dye binding. Anal. Biochem. 1976; 72: 248-254.
22. Whitaker and Bernhard, R. A. (1972). Experments for an introduction to enzymology. The Whiber Press, Davis, Galif.
23. Nidadavolu, H. B.; Metuku, R.P.; Burra, S.; and Pyram,R. (2010). Bioactive compounds from discarded mushroom beds. *Turk J. Biol*. 36: 275-284.
24. Sujimura, H.; Takaja, M.; Katano, K.; Matsumoto, N.; Park, Y. S.; and Okabe, M. (1994). Peroxidase production by carbon and nitrogen sources feed-batch culture of Arthromyces ramosus. *Biotechnol. Lett.* 16:575–580.
25. Zia, M.A.; Kousar, M.; Ahmed, I.; Iqbal, H.M.N. and Abbas, R.Z. (2011). Comparative study of peroxidase purification from apple and orange seeds. *African Journal of Biotechnology*. 10(33), pp. 6300-6303