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DETERMINATION OF OPTIMUM pH OF ACID PHOSPHATASE FROM FRACTIONS PRECIPITATED WITH AMMONIUM SULFATE THE OF PLANTS *ZIZIPHUS*, MYRTLE, LIQUORICE, SEBESTEN OLEANDER, SUGAR CANE, GARLIC SEEDS, ONION, ORANGE FRUIT AND LEMON

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ABSTRACT : Acid phosphatases are metallohydrolases of widespread occurrence and hydrolyse phosphomonoesters at acidic pH in a variety of physiological contexts. The cheak pea seed acid phosphatase (*Cicer arietinum*) enzymes (high molecular weight type glycoprotein), have been previously described, and also we reported acid Phosphatase activity and Optima pH, of Human placenta. In this paper the acid phosphatase activities, the key enzymes from ten plants namely ziziphus, Myrtle, Liquorice, Sebesten Oleander, sugar cane, garlic seeds, Onion, orange fruit and Lemon were purified to homogeneity and determinated those optimum pHs.

Key words : pH, acid phosphatase, plants.

INTRODUCTION

Acid phosphatases or phosphomonoesterases (EC:3.1.3.2) are metalloenzymes and these enzymes found in Human, animal, plants and microorganisem. In mammals, a role of acid Phosphatase activity is ascribed to iron transport (Buhi *et al*, 1982), bone resorption (Hayman *et al*, 1996), antigen presentation and some redox reactions (Hayman *et al*, 2000; 1989). In plants a role of Acid phosphatases is a key role in phosphate acquisition by plants (Vogel *et al*, 2001). Some of rechearcher elaborate on physiology, while other on biochemistry or molecular biology (Duff *et al*, 1994). The animal enzymes also were purified and characterized from bovine spleen (Schenk *et al*, 2000), In humans, plants, Microorganism, yeast they were isolated from pregnant pig uterine fluid (Schlosnagle *et al*, 1974), kidney bean (Yoneyama *et al*, 2004), boar seminal vesicle glands (Wysocki and Strzezek, 2003), Spirodela oligorrhiza (Hoehamer *et al*, 2005), mushroom *Agaricus bisporus* (Guimaraes *et al*, 2004), Human spleen (Aberomand *et al*, 2007), Human bones (Aberomand *et al*, 2011). The present study describes ten optimum pHs about the acid phosphatases form ten plants ziziphus, Myrtle, Liquorice, Sebesten Plum or Assyrian plum, Rose bay, Rose laural, South sea, Oleander rose, sugar cane, garlic, Onion, orange and Lemon.

MATERIALS AND METHODS

Acid phosphatase activity was determined spectrophotometrically at 405 nm by measuring released paranitrophenyl phosphate (at pH 4.8) as substrates (Aberomand and Bhide, 2007) and protein determination was done by Lowry *et al* (1951).

Plant materials

Ten plants ziziphus, Myrtle, Liquorice, Sebesten Plum, Rose bay, Rose laural, South sea , Oleander, sugar cane, garlic, Onion, orange and Lemon (50 g) separately were ground and extracted two times in 100 ml saline (0.85% NaCl) using a homogenizer. The homogenates of plants were mixed with 1-butanol (30ml /100 ml homogenate) and stirred at 4°C for 5 hr. To acetone (total volume) at 20^0C using a polytron blender. The acetone-dried powders were ûltered according to a method of Mohammad Aberomand et al (17). 50 g acetone-dried powers of ten plants were resuspended using a glass homogenizer in 200 ml saline, with stirring for 5-6 hours at 4°C separately. Protein was extracted from the homogenate by centrifugation at 10,000g for 10 min in order to remove solid precipitated. A 30-80% cut contained the majority of the activity and any traces of ammonium sulfate were removed from samples by dialyze bag (Yam *et al*, 1971 and Allen *et al*, 1989). The solution of acid phosphatase was used for Gelfilteration chromatography (Sephadex

Table 1 : The profile of Acid Phosphatase of Ammonium sulfate 80% of plants.

Study plants	Ziziphus	Myrtle	Liquorice	Sebesten	Oleander	Sugar cane	Garlic seeds	Onion	Orange fruit	Lemon
Enzyme activity U/ml	0.18	20.18	28.88	0.517	3.76	9.3	21.17	12.2	5.4	17.66

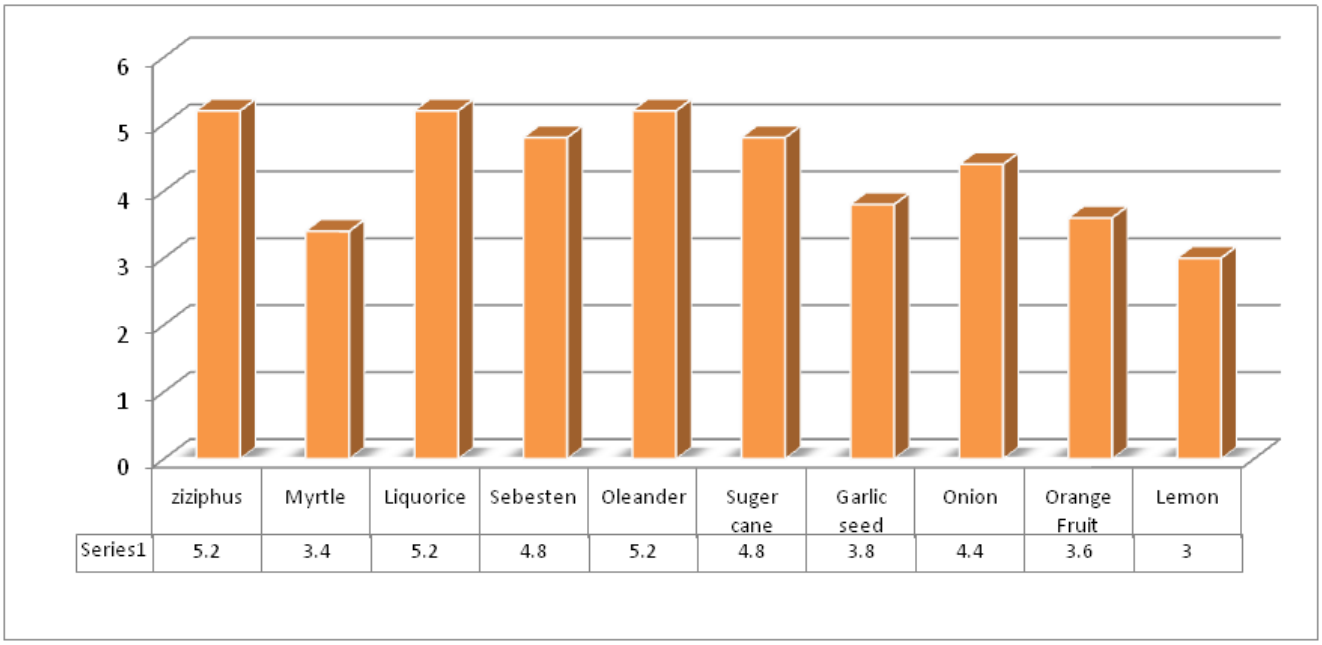

Fig. 1 : The profile of Optimum pH of plants Acid Phosphatase.

G100) (Table 1). Acid phosphatase activity and protein has been determined by method of Mohammad Aberomand *et al* (2007, 2011).

RESULTS AND DISCUSSION

Ten acid phosphatase from ten plants were isolated by gel permeation chromatography and determinated ten different optimum pHs for plants namely ziziphus, Myrtle, Liquorice, Sebesten, Oleander, sugar cane, garlic seeds, Onion, orange fruit and Lemon. The optimum pHs were 5.2, 3.4, 5.2, 4.8, 5.2, 4.8, 3.8, 4.4, 3.6 and 3 repectively. In this study we have identified ten acid phosphatases inhibitors from above ten plants. In this study, inhibitors of all enzymes were compared. Suitable inhibitors are not harmful to Humans and select it for enzyme inhibition patients with cancer. And also reactions of protein phosphorylation and dephosphorylation play a significant regulatory role in cell processes. Activity of many proteins, such as regulatory proteins, histones, permeases of various compounds, and plenty of enzymes, depends on working of protein kinases and phosphatases. Can be used to inhibitors or activators of acid phosphatase,then this enzyme can be inhibited or activated.

CONCLUSION

In previous work, the acid phosphatase activity has been identified and partial characterization (definition of optimum pH) in the Human placenta and some plants as well as chickpea seeds.The acid phosphatase was separated, partial purified from ten plants Ziziphus, Myrtle, Liquorice, Sebesten, Oleander, Sugar cane, Garlic seeds, Onion, Orange fruit and Lemon to homogeneity by chromatography on Sephadex G-200 superfine, and partial biochemical properties (determination of optimum pH) of acid phosphatases were studied. The acid phosphatase from ten plants showed optimal pHs at 5.2, 3.4, 5.2, 4.8, 5.2, 4.8, 3.8, 4.4, 3.6 and 3 with p-nitrophenylphosphate as substrate respectively (fig 1). Ten optimum pHs of acid phosphatases from in ten plants compared ziziphus, Liquorice, and Oleander have same optimum pHs of acid phosphatases. So it seems that these three acid phosphatase from (Ziziphus, Liquorice, and Oleander) have alredy same amino acid sequence in active site. Suggest that three acid phosphatase activity of ziziphus, Liquorice, and Oleander of innibited by same inhibitors.The optimum pHs of acid phosphatases from Myrtle, Sebesten, Sugar cane, Garlic seeds, Onion, Orange fruit and Lemon were different. It can be concluded that the

enzymes of these plants are different isoenzyme and will be inhibite by different inhibitors. According to in the many cancers acid phosphatase increases, Inhibitors can help decrease its activity. In other hand, future resceach, will be plenty of purified enzyme from plants for using in Elisa kit.

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