

# Investigating effect of Amino Acids Leucine, Valine and Alanine on Alkaline Phosphatase Activity of purified Acetone fractions of Sweet Lemon, Garlic and Onion

## Research Article

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## Abstract

Alkaline phosphatase enzyme (ALP, ALKP, ALPase, Alk Phos) (EC 3.1.3.1) produced by plants and microbes is presumed to convert organic phosphorus into available pi, which is absorbed by plants. Considering the importance of Alkaline phosphate enzyme and the balance between its function in the development of various diseases by increasing and decreasing the enzyme level by enzyme activating and regulating compounds, including amino acids and ions, which act as coenzyme and cofactor for the enzyme Can be a major role in Disease improvement. This study aims to comparatively investigate the inhibiting effect of the three Amino Acid as well as Alanine, Leucine and valine on Alkaline phosphatase was which extracted from Sweet Lemon (*Citrus limon* (L.) Osbeck), Garlic (*Allium sativum* L.) and Onion (*Allium cepa* L.). In this study, Leucine, Alanine and Valine amino acids showed strong inhibitory effects 23%, 26% and 34% respectively activity of Alkaline phosphatase obtained saline extract of plants Sweet Lemon, Garlic and Onion.

**Keywords:** Alkaline phosphatase, Leucine, Alanine, Valine.

## Introduction

Alkaline phosphatase (EC 3.1.3.1) exists in animates (animals, plants, microorganisms and yeasts) (1, 2). This enzyme is one of the most ubiquitous enzymes and has been found in yeasts, molds and seeds of some plants, fruits and in many animal tissues such as bovine brain, kidney, liver, spleen, erythrocytes, bones, placenta, hepatocytes and blood plasma (3, 4). These phosphatases catalyze hydrolysis reactions of a variety of orthophosphate esters as well as transphosphorylation reactions in acid medium. It has been characterized and purified from various sources, such as; chickpea seeds (5, 6), pea nuclei (7), bovine brain (8), Medicago truncatula roots (9), mode rarely thermophilic bacterial strain (10), yeast *Yarrowia lipolytica* (11), bovine liver (12) and banana fruit (13). Because this enzyme is a group of enzymes that can catalyze hydrolysis reactions of a variety of orthophosphate esters in acid medium so

it can release phosphate from bones such as tooth or any other bone structure. Consequently, it causes bone disease. Due to important role of enzymes in regulating metabolism and factors affecting its activity can said investigate effective factors in the activity of enzymes as amino acids and ions are necessary to study. The role of amino acids in activating and inhibiting enzymes is that when amino acid concentrations increase in enzymatic environment enzymes, these amino acids can show hydrophobic reaction with amino acids of the apo-enzyme and as a result the active site of the enzyme undergoes changes that affect the activity of enzymes with respect to these statements and causes the enzyme's main activity to be altered and inhibited (12- 15). The aim of this study was to evaluate the effects of leucine, valine and Alanine amino acids on acetone fraction Alkaline phosphatase enzyme obtained from saline extract of Sweet Lemon, Garlic and Onion plants.

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## Materials and Methods

### Chemicals

Sodium azide (Reidel, Germany); Ammonium persulphate (May and Baker Ltd., England); Ethylenediamine tetra acetic acid (E. Merck, Germany), Butanol, Acetone Leucine, Valine, Alanine and dialysis bag were obtained from Sigma Aldrich.

### Partial purification of alkaline phosphatase and effect of amino acids on enzyme

We obtained saline extract of plants Sweet Lemon (*Citrus limon* (L.) Osbeck), Garlic (*Allium sativum* L.) and Onion (*Allium cepa* L.) as first step of partial purification of enzyme. The protein homogenate of antecedently step was mixed with cold 1- butanol and butanol separately. After centrifugation, the resulting precipitate collected and was extracted with 100 ml saline. Since ammonium sulfate (30-80%) precipitates, the obtained precipitate was dissolved in minimum amount of saline and dialyzed against the same till free of ammonium sulfate. The enzyme was incubated with amino acids (30mM) in separate tubes for 1 hour separately, followed by determination of enzyme activity. The protein concentrations and sugar were determined by the method of Lowry (5), (14- 16).

### Enzymatic activity assay

The Alkaline Phosphatase activity was detected using p-nitrophenyl phosphate disodium salt as substrate. In brief, 100  $\mu$ L of the enzyme containing 10  $\mu$ g proteins was added to a tube. 200  $\mu$ L of the substrate (2mM p- nitrophenyl phosphate disodium salt) and 200 mM of citrate buffer (pH 4.6, 0.1 M) were added. Concentrations of each of the compounds of interest are prepared and for each concentration of a test tube, control is considered for all concentrations. To each test tube, 2 ml of saline, 1 ml of buffer with 4.5 pH 0.2 cc Sample containing enzyme and 0.2 cc amino acids are added and all tubes are placed in laboratory conditions for 5 hours. Then to all of them 0.2 cc of the solution 1 mm substrate added and after 2 hours to all tubes added 1 cc NaOH 10%. The light absorption is read at 405 nm wavelengths. It is repeated 3 times for assurance as a result of the test. The p-nitrophenol release was measured by its absorbance at 405 nm (5, 17).

### Ethical principles

This project was approved by the code of ethics of ajums IR.AJUMS. REC.1396.397 in student research committee of Ahvaz Jundishapur University of Medical Sciences.

### Statistical analyses

Statistical analysis was performed with SPSS version 18. Data was analyzed using descriptive statistical methods including mean and standard deviation, and compared by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The significant level was less than 0.05 ( $P < 0.05$ ).

### Results

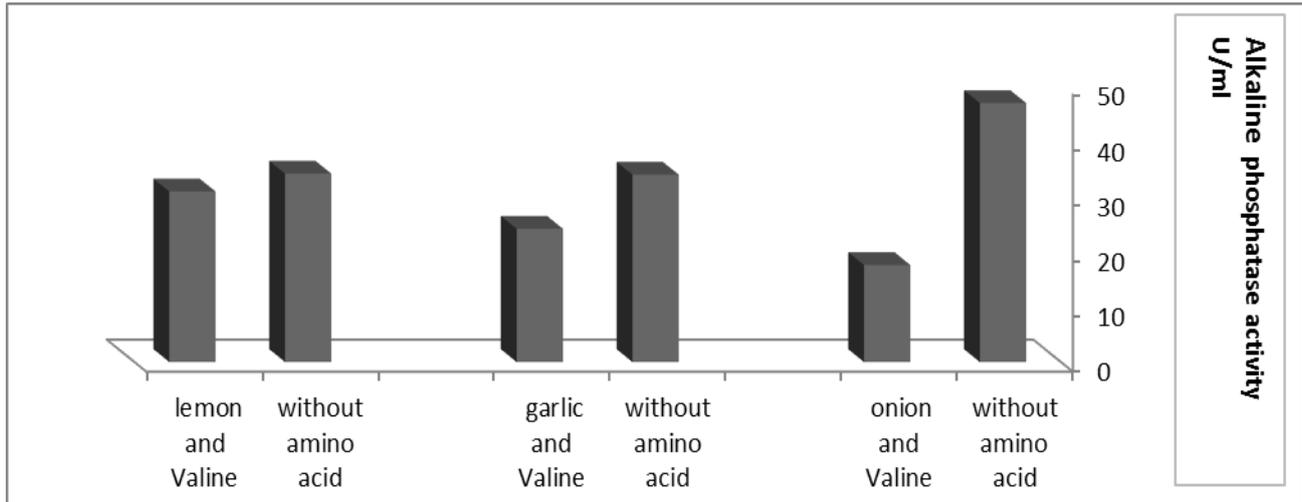
#### Isolation of Alkaline Phosphatase

We had partially purified alkaline phosphatase from plants extract of Sweet Lemon (*Citrus limon* (L.) Osbeck), Garlic (*Allium sativum* L.) and Onion (*Allium cepa* L.) in Table 1. The alkaline phosphatase activity (U/ml), Protein concentration (mg/ml) and Specific activity (U/mg) was shown at Table 1. The enzyme was purified and the final specific activities of Sweet Lemon, Garlic and Onion were 48.2, 73.42 and 76/25 U/mg respectively. Then enzyme incubation with Concentration of 30 mM various amino acid in separate tubes for 5 hours, followed by determination of enzyme activity was achieved. The results are showed that Leucine, Alanine and valine had strong inhibitory effects on enzymatic activity. The inhibiting effect of the three amino acids Alanine, Leucine and Valine on the activity of alkaline phosphatase was determined. All three tested amino acid inhibited the activity of alkaline phosphatases. In alkaline phosphatase activity of Lemon, Alanine showed the most inhibiting value 14/71% and followed by Valine 9.48% but leucine increased enzyme activity to 4.07%. In alkaline phosphatase activity from Onion, Valine showed the most inhibiting value (62.56%), followed by Alanine and leucine have inhibition 53.56 and 53.45 respectively also in alkaline phosphatase activity from Garlic, Alanine showed the most inhibiting value (49.95%), followed by Valine and Lucien have inhibition 26.24 and 22.15 respectively. The results also showed in Figures 1, 2 and 3.

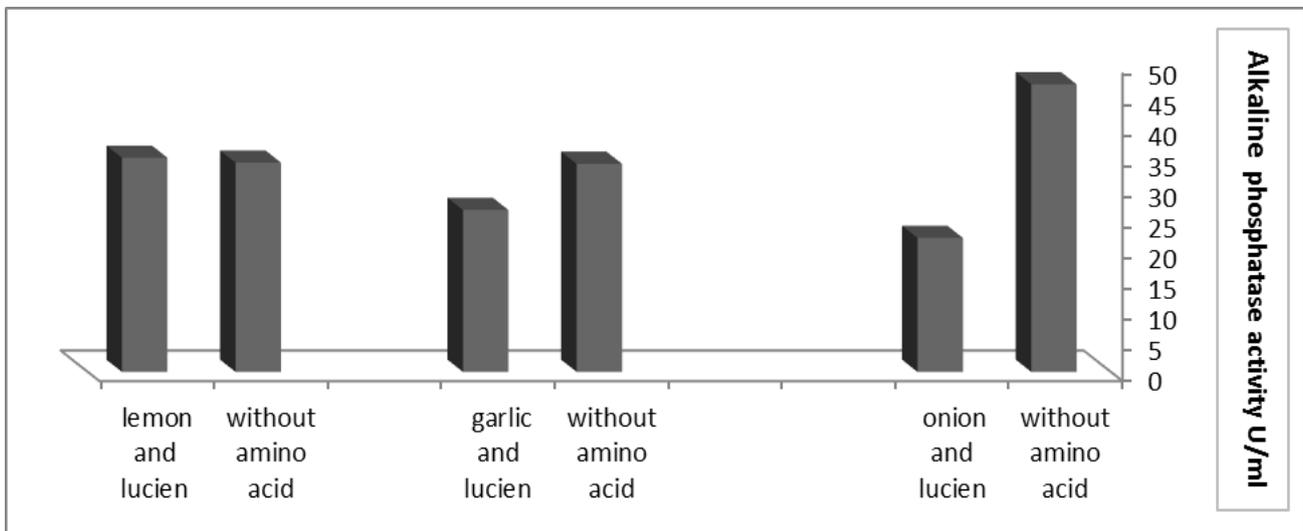
**Table 1: Partial Purification steps of alkaline phosphatase of plants, Sweet Lemon (*Citrus limon* (L.) Osbeck), Garlic (*Allium sativum* L.) and Onion (*Allium cepa* L.).**

Plant	Step	alkaline phosphates activity U/ml	Protein concentration mg/ml	Specific activity U/mg
Sweet Lemon	Acetone extract	29.52	1.41	20.9
	Ammonium sulphate extract	17.96	0.374	48.2
Garlic	Acetone extract	23.94	1.24	19.3
	Ammonium sulphate extract	25.7	0.35	73.42
Onion	Acetone extract	24.32	0.52	46/7
	Ammonium sulphate extract	12.2	0.16	76/25

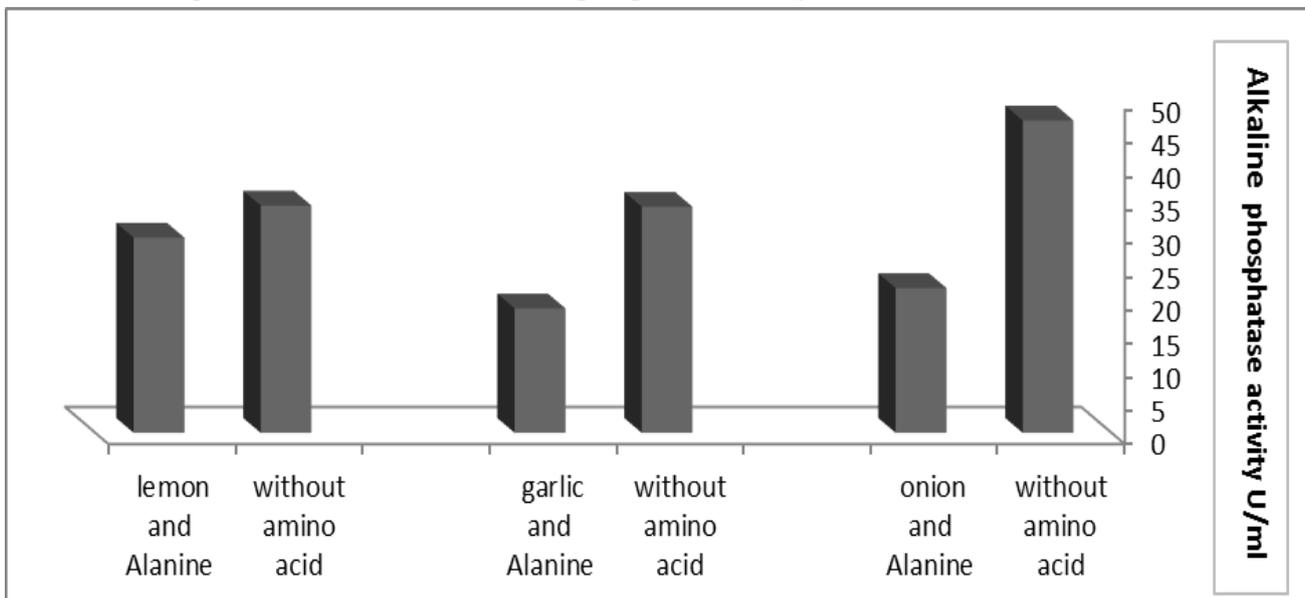
**Fig. 1: Effect of Valine on alkaline phosphatase activity of Lemon, Onion and Garlic.**



**Fig. 2: Effect of Leucine on alkaline phosphatase activity of Lemon, Onion and Garlic.**



**Fig. 3: Effect of Alanine on alkaline phosphatase activity of Lemon, Onion and Garlic.**



## Discussion

The measurement of alkaline phosphatase enzyme is recommended as a routine screening test for the diagnosis of various diseases, including cholestasis, biliary obstruction, obstructive jaundice, cysts and liver abscesses and bone marrow metastases (18). This study examines the effect of amino acids of Leucine, Valine and Alanine on alkaline phosphatase activity. The alkaline phosphatase purified of plants Sweet Lemon (*Citrus limon* (L.) Osbeck), Garlic (*Allium sativum* L.) and Onion (*Allium cepa* L.) by ammonium sulphate precipitation and Dialysis bag. Then it was affected by amino acids. The results show the enzyme activity was inhibited by the polyamines (amino acids) as Valine, Alanine and Leucine. Considering that the increase of this enzyme in the body is associated with the onset of mitosis, cholestasis, biliary obstruction, obstructive jaundice, cysts and liver abscesses, bone marrow metastases, germinal vesicle fracture also increasing the activity of the enzyme as a biochemical marker for inadaptation to hepatocellular factors (18, 19, 20). In addition, decreasing activity alkaline phosphatase enzyme by removing the phosphate from the pathways of the glycogen metabolism pathway leads to a change in the activity of these enzymes and can make sure that the metabolism of glycogen is threatened and lead to liver disease. For this reason, we decided to study the effect of amino acids of valine, Alanine, Leucine on alkaline phosphatase activity. So, if enzyme activity decreased under effect of amino acids, the amino acids acts as inactivator of the glycogen metabolism pathway and cholestasis, bone marrow metastases and germinal vesicle fracture (21, 22, 23). In this study, the inhibitory effect of valine was found to be higher than the other. It can be concluded that valine inactivates the alkaline phosphatase enzyme in the mentioned diseases. Therefore, in the future, the active ingredient of the Alkaline phosphatase inhibitor Valine can be given as a medicine, and it can be given to the patient during the treatment of the diseases under the supervision of the physician, thereby increasing the Control of activity of the Alkaline phosphatase in the cell and serum.

## Conclusion

We conclude that during the treatment of diseases, the amount of activity of the alkaline phosphatase enzyme should be monitored. Thus, the amino acids as activators or inhibitors metabolic pathway are necessary for Human. Also Leucine, Alanine and Valine can be used as inhibitors alkaline phosphatase activity against its high level in patients.

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## Conflict of interest

There are no conflicts of interest in this study.

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