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A Study of Anticancer Activity for Partial Purified Urease Isolated from *Lagonychium farctum* Plant

Mohammed Hasan Allawi* and Mohamed Qais Abed
Department of Biology, Collage of Science, University of Anbar, Anbar-Iraq.
* Corresponding Author: m337799h@gmail.com

Abstract

This study included two experiments, the first of which included the investigation of the urease enzyme in the plant extract of the *Lagonychium farctum*, which included the extracts of the seeds, leaves and stems the plant. The results, showed that the highest enzymatic and quality efficacy of urease was in seed extract of the *Lagoonchium farctum*. The optimal conditions for enzyme extraction were determined using different solution, pH and temperature. The Potassium Phosphate Solution at 0.02 µl pH 8 was the homogenous enzyme extract, with an enzymatic concentration of 3.195 U/ml. The optimum reaction time for enzyme extraction was 30 min, giving an enzyme activity of 3.274 U / ml. The optimum reaction time for enzyme extraction was 30 minutes where it gave enzymatic efficacy of 3.274 U/ml. The purification was by several steps including ammonium sulphate deposition with different precipitation ratios. The optimum saturation was 50 %, followed by ionic exchange on the DEAE-cellulose column and gel filtration on the Sephadex G-200 column. The purification time was 29.6 and the enzyme yield was 75 %. The second experiment included study of the effectiveness of the enzyme Urease as an Anticancer activity. Results showed that the urease enzyme had an inhibitory effect on the breast cancer line MCF7; it has been shown to be effective in the direction of breast cancer when the concentration of the partially purified enzyme is increased while in contrast to the natural cell line WRL68. [DOI: 10.22401/ANJS.00.1.13]

Keywords: Urease, *Lagonychium farctum* Plant, Purification, MCF7, WRL-68 and Anticancer.

Introduction

Urease is amidohydrolase, a nickel-based metal enzyme (Ni^{+2}) to stimulate the hydrolysis of urea at a rate of 1014 times faster than the non-stimulating reactions producing ammonia and carbon dioxide, [1,8]. Urease produce a large number of organisms, including high-end plants and microorganisms, it is of great importance in biotechnology, clinical chemistry, immunochemistry and medical fields, because of its association with some diseases that affect humans such as: diseases of the urinary system Gastric ulcer, and duodenal ulcers, which develop if not treated with cancer, [9].

Cancer has emerged as one of the leading causes of death in the world, It is a serious disease that kills the lives of millions of people every year, One of three women is injured, while one in four of them dies, In 2005, the American Cancer Society estimated its deaths in the United States to be more than 570.000, [2]. The study of efficacy Anticancer of Urease enzyme gave it significant medical outcome, The urease enzyme is a Chemotherapy agent for types of human cancers such as breast cancer and lung cancer, In addition to its use as Antibacterial, [2]. Therefore, the present study aimed at: Investigation of the presence of urease enzyme for some plant sources belonging to the legume family, Purification of the enzyme using available chromatographic methods such as Ion Exchange Chromatography and Size Exclusion Chromatography, Detection of amino acids in the purified enzyme using a device amino acid analyzer and Study the effectiveness of urease enzyme as Anticancer The direction of the breast cancer line.

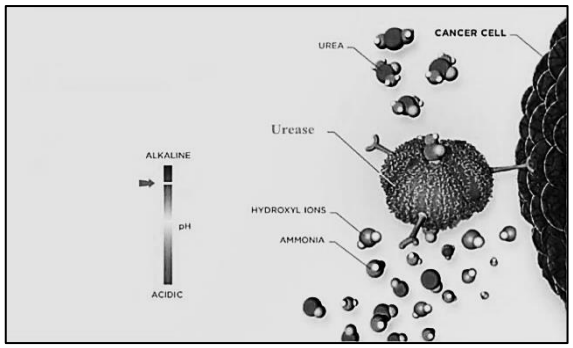


Fig.(1) Urease enzyme activity inhibition cell line.

Materials and Methods

The current study included the selection of a number of seeds of the legume family plants for the purpose of obtaining urea enzyme. The most efficient plant is chose in terms of enzyme efficacy and then the enzyme is extracted, purified and characterization and the study of the optimal conditions for the effectiveness of Urease and after the test was conducted anti-cancer effectiveness *in vitro.*

Seeds preparation

The seeds were placed in the Petri dishes containing the filtration papers moistened with distilled water at a temperature of (32 ^{o}C) until reaching the stage of germination to obtain the highest enzyme efficacy at this stage, [3]. The developing seeds were weighed at 50 gm and tested with 100 ml of the solution Potassium phosphate buffer PH=8 and centrifuged quickly 11000 rpm for 30 min., Discard the precipitate and leachate collection as a crude Extract to estimate the effectiveness of the urease enzyme.

Enzymes assay

The method mentioned by Achakzai *et al* (2003) was followed. In measuring the activity of the enzyme on converting one micromol from urea to ammonia.

Purification of Urease enzyme

Urease enzyme purified more than once using different purification methods including Precipitation with ammonium sulfate at saturation ratio 50 %. Then purification with Ion exchange chromatography by using a column DEAE-Cellulose and use Gel filtration chromatography (Sephadex G-200).

Anticancer activity of Urease enzyme

The study was carried out in the laboratory outside the body of the in vitro to join the purified anti-cancer efficacy of urease on the cancerous line (MCF7) According to the method [10]. To determine the toxicity of the enzyme on the cells line MCF7, The cell line culture was incubated with different concentrations of the enzyme (1.6, 0.8, 0.4, 0.2, 0.1, 0.05 and 0.25) and µg/ ml. Compression rate calculated for the growth of cancer cells Per concentration as well as calculate the percentage of cell vitality as indicated in, [11].

Results and Discussion:

Detection of Urease in plant parts of *Lagonychium farctum*

The efficiency of Urease was detected in parts of *Lagonychium farctum* by extracting urease from seeds, leaves and stems using a homogenization method with 0.02 mL Potassium phosphate solution. The highest enzymatic activity recorded was (10.931) units/ ml and the efficacy of (1.521) units/mg of urease was in the extract of seeds, while the enzymatic activity and the specific efficacy in the extract was 1.263 units/ml and 0.143 units/ respectively. The enzymatic and quality activity of the chickpea plants was 1.145 units/ ml and 0.143 mg/mg. The choice of the appropriate concentration of the solution is necessary in the separation methods to avoid the effects of ionic power is not specialized, [13,16].

Purification of Urease extracted from seeds of *Lagonychium farctum*

Urease was purified from the *Lagonychium farctum* plant extract using various chromatography techniques, including ion exchange chromatography and gel filtration after ammonium sulphate deposition. The results indicated in Fig.(2) show that the best enzymatic efficacy was at saturation rate (50%), with (15.29) U/ml compared with raw extract.

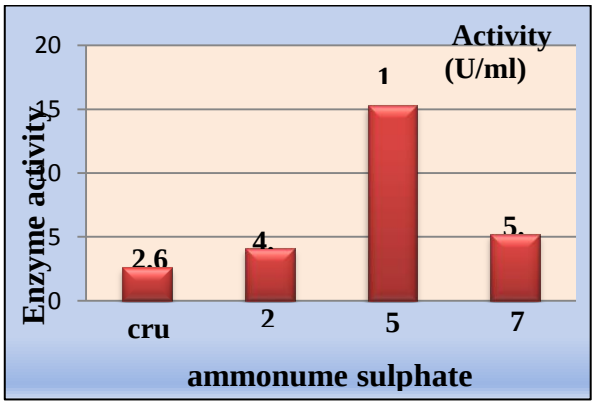


Fig.(2) Determination of the optimum saturation ratio of ammonium sulphate in the concentration of urease enzyme extracted from plant seeds Lagonychium farctum.

Purification Using Ion Exchange Chromatography

Purified Urease was extracted from the seeds *Lagonychium farctum* Ion Exchange Chromatography (DEAE-Cellulose), This technique has been used for its high capacity for dynamic separation, High capacity to bind protein in addition to easy to achieve, [4,13]. Based on these results, use the ion exchanger, Take 8 ml of the crude extracts containing the concentrated urease enzyme in sucrose on DEAE-Cellulose. The column was then washed and calibrated with a volume of emergency solution potassium phosphate; related proteins were recovered using a saline gradient sodium chloride.

Table (1)
Protein concentration, enzyme activity Quality effectiveness of extracts in different parts of the Lagonychium farctum plant.

Plant part	*Volume ml*	*Enzyme activity Unit / ml*	*Protein concentration mg / mL*	*Quality effectiveness Unit /mg protein*	*Overall effectiveness Unit*
Seeds	50	2.613	10.654	0.250	130.65
leaves	50	1.263	8.813	0.143	63.15
Stems	50	1.145	8.173	1.40	57.25

The results indicated in the Fig.(2) showed the presence of one protein peak in the washing phase While we note the emergence of four protein peaks after recovery with saline gradient. All these protein peaks were detected in the wash and recovery phase by measuring absorbance at a wavelength of 280nm for each separate fraction. Enzyme activity was detected in the four protein peaks. The results showed that the third summit at separate parts (53-55) recorded an enzyme activity of urea of 8.98 U/ ml. In another study, the ion exchanger DEAE-Cellulose was used to purify urease from the dandylifera nucleus, giving 256.6 mg / ml protein and 51.8 %, [5].

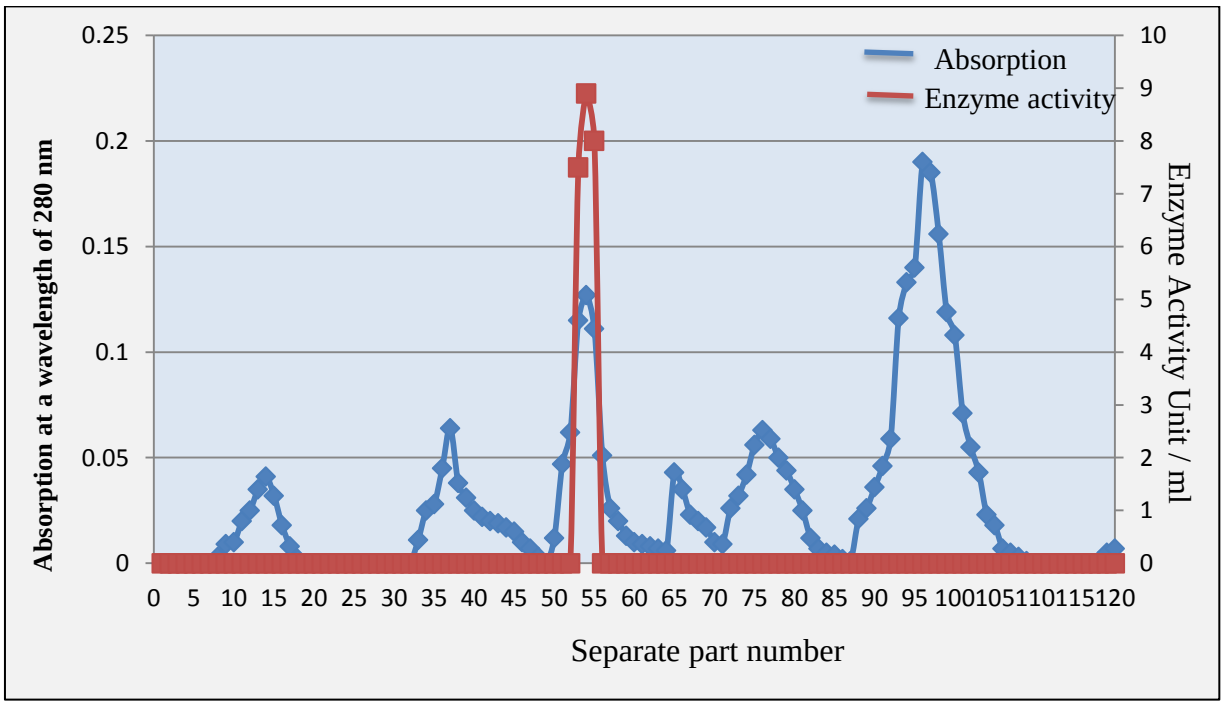


Fig.(3) Ion exchange chromatography to purify the urease enzyme using the ion exchanger DEAE -Cellulose with a distance of 1.25×18 cm.

Purification using gel filtration chromatography

The next step was to purify the urease from the *Lagonychium farctum* seeds after the ion exchange step, loaded 10 mL of partially purified enzyme to the Sephadex G-200 column with dimensions of 32×1 cm, previously balanced with solution potassium phosphate. The Sephadex G-200 has separation limits within the range (5000-600000) Dalton, which allows the ability to separate with a high degree of purit, [9,11]. The proteins eluted during the column at a flow rate 1 ml / min and collected at 5 ml/ fraction. Protein peaks were detected by measuring the optical density at 280 nm wavelength using ultraviolet light, [7].

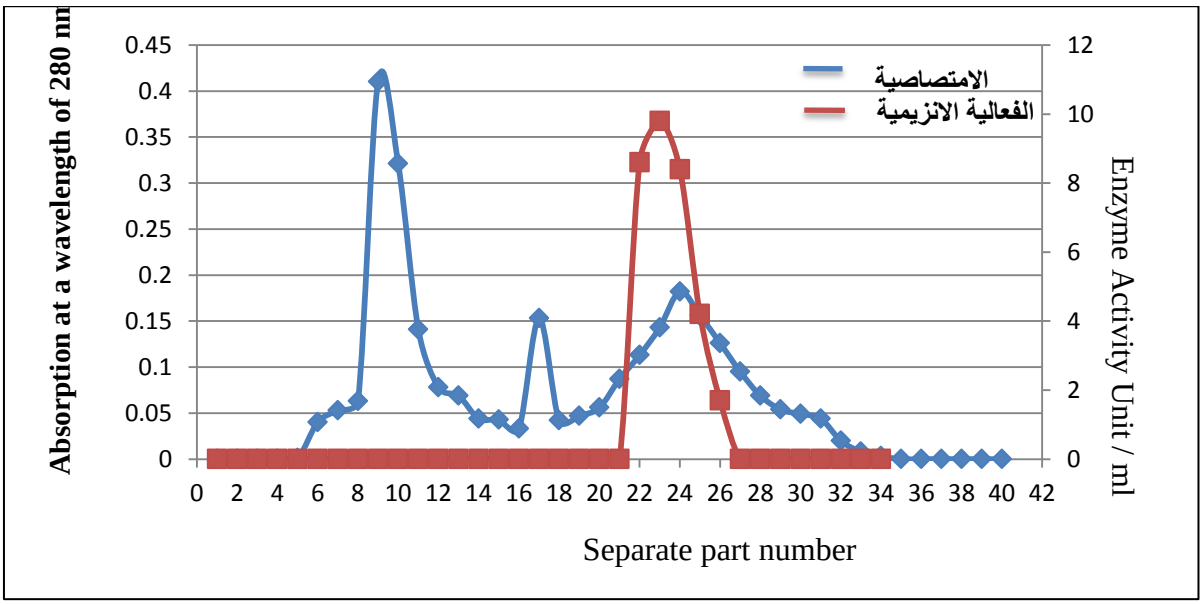


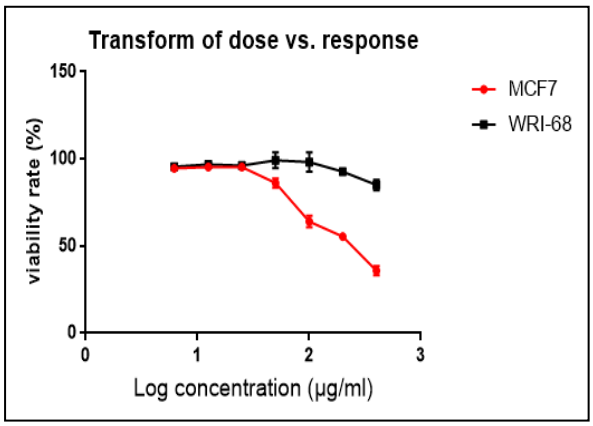
Fig.(4) Chromatography of gelatinous filtration of urease extract from Kharnub plant using G-200 sulfide gel column with dimensions (32X1) cm

Determination of molecular weight using SDS-PAGE

We used the method described by Laemmli (1970) in estimating the molecular weight of purified urease from the seeds of the *Lagonychium farctum* plant, The results show with molecular weight of the urease enzyme is approximately 85 kDa. The diffrent molecular weight of the urease because of the variation in the number of constituents of the enzyme, [6].

The inhibitory activity of the urease enzyme towards the cancer line

The anti-cancer cell effectiveness for the urease enzyme partially purified from the seeds of the *Lagonychium farctum* to perform viability testing in the MTT assay method. The results showed an inhibitory efficacy of urease against cancer cell line MCF7 isolated from cancer cells for breast cancer as compared to normal cells of liver cells in human WRL-68 as shown in Fig.(6). As demonstrated by the study of anti-cancer effectiveness the use of different concentrations of the enzyme (1.6,0.8,0.4,0.2,0.1 and 0.25) µg/ml Urease affects its inhibitory activity by increasing concentration by 35.84 %, 55.48 %, 64.12 %, 86.23 %, 95.37 % respectively where the proportion of living cells of MCF7 cancer cells decreased. While the same results were shown when using the same concentrations on normal cells (WRL-68). The effect on cell vitality was not more than 14.97 % and at the highest concentration of 1.6 µg/ ml when low concentrations were used; there was no apparent effect on the vitality of normal cells. Used cytotoxicity tests with many biomedical and environmental applications. Therefore it was selected for determination of Urease cytotoxicity, [15]. Some studies have indicated the role of urease in inhibiting cancer cells by producing ammonia and thus converting the surrounding medium to alkaline [14,15].



Conclusions:

- The seeds of the *Lagonychium farctum* plant can be counted as the first non-traditional land source for Urease production.
- The amnion acids analysis of the urease enzyme is important in the detection of amino acids and their concentrations.
- The urease enzyme has a toxic effect towards the line of breast cancer cells based on concentration.
- he acquisition of urease enzyme is an inhibitory effect towards antibacterial cells

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