CHEMOINFORMATIC ANALYSIS OF SOME FUNGAL PECTINASES INHIBITORS

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ABSTRACT

Fusarium oxysporum f. sp. lycopersici attacks tomato plants and causes wilt disease. Fusarium Pathogenicity is including pectinases enzymes which enable the Fungal penetration into host cell wall.

The present study is focused on using Computational tools such as Auto-Dock program for screening of inhibitors of endo and exopolypgalacturonase enzymes. It based on Lamarckian Genetic Algorithm (LGA) that estimate the binding energy and inhibition constant as parameters to select the best binding. The binding energy and amino acids interactions for the selected inhibitors were compared with that of the enzyme substrate (polygalacturonic acid) Allium species such as onion plant have been used widely as antimicrobial and antifungal plants. It contains ranged between 1 and 5 % of non-protein sulfur amino-acids, including S-E-Prop-1-enyl-L-cysteine S-oxide, S-3-Allylsulphinyl-L-alanine and S-Methylcysteinesulphoxide have satisfactory binding interactions and inhibition constant with endo and exopolypgalacturonase. In the present study, these compounds were extracted from white onion bulb Giza 20 and detected in the onion extract LC/MS analysis. The Inhibitory effect of these compounds for exopolypgalacturonase enzyme was confirmed experimentally by determination of the enzyme activity in the presence and the absence of these compounds. White onion extract 45% inhibition percentage of the exopolypgalacturonase activity. The enzyme kinetic study showed increase in the Km value with stable V-max value in presence of 7µg/µL of the onion extract. Also, In-vitro experiment of inhibition of F. oxysporum growth in presence 20% and 40% of onion extract showed inhibition percentages of 47% and 53% respectively. The results concluded that onion extract inhibits Fusarium growth through inhibition of exo and endo polygalacturonase. The inhibitory effect of onion extract could be due to its contents of S-E-Prop-1-enyl-L-cysteine S-oxide, S-3-Allylsulphinyl-L-alanine and S-Methylcysteinesulphoxide, these compounds have excellent binding interactions and inhibition effects on both exo- and endopolypgalacturonases enzymes of Fusarium oxysporum f. sp. Lycopersici.

Keywords: Chemoinformatic, Auto-Dock program, Fusarium oxysporum, Pectinases, Exopolypgalac- turonase; Onion extract

INTRODUCTION

Tomato (Solanum lycopersicum L., syn. Lycopersiconesulentum Mill.) is one of the most wide- spread vegetable crops worldwide. Tomato worldwide production is around 115.95 million tons per year. However, the economic production of tomato crop is affected by various biotic and abiotic stress conditions (Bergougoux, 2014; Gupta and Rashotte, 2014). Vascular wilt disease is caused by Fusarium oxysporum f.sp. lycopersici (FOL). It is a soil borne pathogen in the class Hyphomycetes that causes wilt of tomato that may cause 10-90% loss in the yield (Rai et al 2011; Singh and Kamal 2012).
The fungus attacks the plant with its sporangial germ tube or mycelium by infecting the plant’s roots. The roots can be infected directly through the root tips, wounds or at the formation point of lateral roots. The fungal mycelia enter the xylem vessels branches and produce micro-conidia, which are carried upward in the sap stream. The fungal growth blocks the plant vascular tissue so that the water supply is greatly affected. This lack of water induces the leaves tomato to close; the leaves wilt and the plant eventually dies (Ma et al 2013). The control of vascular wilt disease is mainly achieved through the use of chemical fungicides (Minton 1986). Fusarium can be a human pathogen, a human pathogen and a toxin producer.

Pectinases are group of hydrolytic enzymes that cause pectin degradation by various mechanisms. The pectinases family includes protopectinases, polygalacturonases, lyases and pectin esterases. Polygalacturonase (PG) is the first cell-wall-degrading secreted enzyme from phytopathogenic fungi (Garcia et al 1997).

Fusarium oxysporum polygalacturonases enzymes are divided into two groups:
1) Endopolygalacturonases (E.C.3.2.1.15) that breaks the polymer chain in a random pattern liberating oligogalacturonides and galacturonic acid, 2) Exopolygalacturonases (EC 3.2.1.82) that cleaves the polymer bonds releasing one galacturonic acid residue from the non-reducing end (Garcia et al 1997).

Chemoinformatic is widely used in drug discovery (Srinivasan et al 2014) and in silico prediction of interactions between small molecules and proteins (Manly et al 2001; Schoichet, 2004 and Koppen, 2009). There are many software packages available for the conduct of molecular docking simulations like, Auto-Dock, GOLD (Collignon et al 2011).

Auto-Dock 4.2 program is the most recent version which has been widely used for virtual screening, due to its enhanced docking speed (Schames et al 2004). It is based on Lamarckian Genetic Algorithm (LGA) that estimate the binding energy and inhibition constant as parameters to select the best binding.

Allium species contain ranged between 1 and 5 % of non-protein sulfur amino-acids (Lancaster and Shaw, 1989). One class of these secondary metabolites it is called S-alkenyl-L-cysteine sulphoxides that gives the characteristic flavor associated with the Allium species. Sulphoxide compounds found in the Allium species: include S-E-Prop-1-enyl-L-cysteine S-oxide, S-3-Allylsulphphnly-L-alanine and S-Methylcysteine sulfoxide (Lancaster and Shaw, 1989). Onion plant have been used widely as antimicrobial and antifungal (Hasan et al 2005).

The aim of the present study is to computational investigation using Auto-Dock program was carried out to screen the inhibitors of endo and exopolygalacturonase enzymes. Also, evaluate the ability of onion extract to inhibit fusarium oxysporium growth and their endo and exo polygalacturonase.

MATERIALS AND METHODS

Group of database and software has been used to visualize the binding between inhibitors and enzymes and Chemical structure comparison for the selected inhibitors to identify the active site in selected compounds that given most attachment software’s which are showed interaction including Cgwin (a data storage) c:/program and Python 2.5 were simultaneously downloaded from www.cygwin.com, Molecular Graphics Laboratory (MGL) tool and Auto-Dock 4.2 were downloaded from www.scripps.edu. Discovery studio visualizer 2.5.5 was downloaded from www.accelerys.com where it was used to show the interaction and comparison between enzyme and inhibitor (Syed et al 2013). BRENDA enzyme databases used to knowing the amino acids sequence, Pubchem database used for known 3D structure of chemical compound, I-TASSER (Iterative Threading Assembly Refinement) databases used for prediction of 3D structure of enzyme, National Center for Biotechnology Information (NCBI) used for knowing the domain for enzyme. From Chemoinformatics search for several compounds against endo-and exo- polygalacturonase of Fusarium oxysporum f. sp. Lycopersici fungs. A group of different compounds including alkaloids, Flavonoids, Terpenoids, sulfur compounds and phenols were used as inhibitors to several pathogenes.

We had used the Lamarckian Genetic Algorithm (LGA) for ligand conformational searching, which is a hybrid of a genetic algorithm and a local search algorithm. It is depended on free-energy scoring function for calculation the binding energy (Madeswaran et al 2012). We were used PDB format that described as PDBQT file was used for coordinate files which includes atomic partial charges. Auto-Dock program was used for preparation PDBQT files from PDB files (Khodade et al 2007).
Formation of PDB files for target protein with the Auto-Dock program involved adding all hydrogen atoms to the macromolecule, which is a step necessary for correct calculation of partial atomic charges. Gasteiger charges are calculated for each atom of the macromolecule in Auto-Dock 4.2 and Kollman charges is calculated to ligand structure, we had used Three-dimensional of size 60 × 60 × 60 Å with 4.58 Å AutoDock 4.2 can calculate the desolvation energy (electrostatic energy, Vander Waals energy), torsion energy which was resulted from attachment between ligand and protein (Konc et al 2011). Auto-Dock program was used to analyze the results as parameter the binding site, binding energy and inhibition constant to enzyme inhibition prediction (Park et al 2006).

Pectinases enzyme production and extraction of inhibitors

Pectinases enzymes were produced in liquid medium by Fusarium oxysporum f. sp. lycopersici. The medium has the following composition: 0.2 g MgSO$_4$.7H$_2$O, 0.4 g K$_2$HPO$_4$, 0.2g KCl, 1g NH$_4$NO$_3$, 0.01 g FeSO$_4$, 0.01 g ZnSO$_4$, 0.01 g MnSO$_4$ per liter. Cultures were supplemented with 1% Pectin. The flasks were maintained at 120 rpm on a shaker for five days (Garcia et al 1997). S-alkenyl-L-cysteine sulfoxides compounds.S-E-Prop-1-enyl-L-cysteine S-oxide, S-3-Allylsulphinyl-L-alanine and S-Methylcysteine sulfoxide from the white bulb onion (Giza 20), were extracted by adding 200g of homogenized onion bulb to a mixture of methanol: chloroform: water (12:5:3) containing 10 mM hydroxylamine (Edwards et al 1994). The mixture was partitioned between chloroform and water and the methanol water layer was kept, dried under vacuum at 40°C and resuspended in 5ml distilled water.

LC/MS analysis

Main profile of the active ingredients of onion extract was detected by HPLC mass scan to identify two molecular mass (178 and 152) according to Yukihiro et al 2017. Onion extract was analyzed by Acquity UPLC/MS/MS (Waters) system, equipped with Xevo TQD MS spectrometer with an ESI+ source under conditioning were Cone gas flow (L/H) desolvation gas flow 900°C, source temperature 150°C and collusion energy was 10.

Exopolygalacturonases (EC 3.2.1.82) enzyme inhibition assay in absence and presence of onion extract inhibitor

The Exopolygalacturonases was determined by measuring the release of reducing sugars by 3,5-dinitrosalicyclic acid (DNS) method according to Saleh et al 2009 as explained in Table (1).

| Reagent                | Experiment 1* | Experiment 2* |
|------------------------|---------------|---------------|
| Substrate solution*    | 500µl         | 500µl         |
| Buffer solution*       | 570 µl        | 550 µl        |
| Inhibitor solution     | --            | 20 µl         |
| Enzyme solution        | 200µl         | 200µl         |

*substrate solution composed of 0.1 % polygalacturonic acid in 0.1 M acetate buffer pH 4.2.
*Buffer solution is0.1 M acetate buffer pH 4.2.
*Experiment 1: is the enzyme activity in absence of the inhibitors.
*Experiment 2: is the enzyme activity in presence of the inhibitors.

The tubes were incubated for 1h at 40°C followed by addition of 3ml DNS solution then boiled at 100°C for 10min. The control tubes were prepared by the same manner without incubation time to avoid any enzyme activity.

The absorbance was measured at 540nm using spectrophotometer. A standards curve was prepared from different concentrations (0.02 – 0.1 g/100 ml) of D-galacturonic acid in buffer solution. One unit of the enzyme was expressed as the amount of enzyme that releases one mg of galacturonic acid/ml/min.

% inhibition = [(No. of enzyme units in absence of inhibitor - No. of enzyme units in presence of inhibitor)]/ (No. of enzyme units in absence of inhibitor)]*100

Kinetic study

Kinetic study of inhibition was determined using Lineweaver–Burk equations (Jenny et al 2014). This study was carried out by different substrate concentrations (mg/ml) 0.04, 0.08, 0.16, 0.24, 0.32.

The enzyme assay was performed as explained in the assay method. The used inhibitor solution concentration was 7 mg/ml. The relationship of the 1/V values (where “V” is the No. of enzyme units/ml) versus 1/S (where “S” is the substrate concentration).
The values of kinetic parameters (Km, Vmax,) were determined from the Lineweaver-Burk plot. The inhibition mode was determined from the intercept of Y axes that explains the 1/Vmax. (Zhao & Kim., 2004 and Won et al 2007).

In-vitro evaluation of F. oxysporum inhibition by onion extract

Volume of 100ul from two different concentrations of the onion extract solution (20%, and 40%) were added in wells in Petri dishes containing sterilized potato dextrose agar medium (PDA) and inoculated with a disk from the fungus. Plates without plant extract served as negative control. Plates were incubated at 27°C. Radial growth of mycelium was measured after seven days of incubation according to Anil and Raj 2015. The results were compared with negative control. The inhibition percentage was calculated according to Maurhofer et al 1995

RESULTS AND DISCUSSION

Prediction of 3D structure of endopolygalacturonase enzyme EC 3.2.1.15 of Fusarium oxysporum f.sp. Lycopersici

The 3D modeling for endopolygalacturonase enzyme EC (3.2.1.15) of Fusarium oxysporum f. sp. lycopersici was obtained from TASSER Database. The domain of this enzyme was identified from NCBI database. It was Glyco hydro_28 in range from 40 -369 amino acid shown in Fig. (1). The 3D Structure of Sodium Polygalacturonate (substrate) was obtained from pubchem database. The active site and interaction between 3D modeling of endopolygalacturonase and 3D structure of sodium polygalacturonate was illustrated in Fig. (2). The group of amino acids responsible for this interaction are: K241, T215, S237, G236, K266, D210, D209, N186, D188, H149, D124, N117, S115, A213, H125, N80, D81. This was illustrated in Fig. (3). The types of organic compounds and their sources, inhibition constant, binding energy (ΔG) and binding sites, which had been used in docking to search about nature of organic compounds that have the same amino acid binding site as compared with the substrate are explained in Table (2). It was found that these compounds didn’t have the same binding site as compared with the substrate and it has low binding energy and high inhibition constant. The lower binding energy (ΔG) value is the stronger binding between the inhibitor and the enzyme. The inhibition constant indicates the lowest inhibitor concentration that inhibits halve of enzyme velocity. Data in Table (3) showed types of active compounds in the onion plant, inhibition constant, binding energy ΔG and binding sites of these compounds with endopolygalacturonase enzyme. These compounds have similar amino acids group in the interaction site, binding energy and inhibition constant as compared with substrate for the same enzyme. These compounds are S-E-Prop-1-ethyl-L-cysteine S-oxide, S-3-Allylsulphinyl-L-alanine and S-Methylcysteinesulfoxide. The interaction with S-3-Allylsulphinyl-L-alanine compound has the lowest inhibition constant and binding energy Fig. (4).

Prediction of 3D structure for exopolygalacturonase enzyme EC3.2.1.82:

The 3D modeling of exopolygalacturonase enzyme EC (3.2.1.82) of Fusarium oxysporum f.sp. lycopersici obtained from TASSER Database search and from NCBI database. The Domain of exopolygalacturonase enzyme is Glyco_hydro_28 in range from 40 - 425 amino acid shown in Fig. (5). The active site and interaction between 3D modeling of endopolygalacturonase and 3D structure of sodium polygalacturonate was illustrated in Fig. (6). The interaction amino acids group is: K314, Y349, S283, K230, K260, D255, H277, W201, D233, N231, D254, Q286, Q308, K230, L238, S258, F204. ΔG is -2.4 kcal mol\(^{-1}\) and inhibition constant is 16.4mM Fig. (7).

Data in Table (4) show types of active compounds in onion plant, inhibition constant, binding energy ΔG and binding site of these compounds with exopolygalacturonase enzyme. They have similar amino acids in the interaction site, binding energy and Inhibition constant as compared with the substrate. These compounds are S-3-Allylsulphinyl-L-alanine, Methylcysteine sulfoxide and S-E-Prop-1-ethyl-L-cysteine S-oxide, which showed lowest binding energy and inhibition constant Fig. (8).

Comparison between substrate and inhibitor

S-E-Prop-1-etyl-L-cysteine S-oxide compound was the best inhibitor in onion extract because it gives lowest inhibition constant and lowest binding energy with endo and exopolygalacturonase compared with other inhibitors. The 3D structure of sodium polygalacturonate (substrate) Fig. (9) and 3D structure of S-E-Prop-1-etyl-L-cysteine S-oxide
Fig. 1. 3D structure of (Glyco_hydro _28) Domain for endopolygalacturonase enzyme

Fig. 2. The overall 3D view of endopolygalacturonase interact with substrate in the active site
Fig. 3. The binding site resulted to hydrogen donor and acceptor for endopolygalacturonase enzyme with substrate was K241.T215.S237.G236.K266.D210.D209. N186. D188.H149.D124.N117.S115.A213.H125.N80.D81.

Fig. 4. Binding site resulted to hydrogen donor and acceptor for S-3-Allylsulphinyl-L-alanine with endopolygalacturonase was K241.T215.S237.G236.K266.D210.D209.N186. D188. R264.D191. H231. Y299.A213.

Fig. 5. 3D structure of domain (Glyco_hydro_28) for exopolygalacturonase enzyme.
Table 2. Comparative evaluation of Inhibition constant- binding energy and binding site of some pectinase inhibitors (terpenoides, alkaloids, flavonoides, phenolic and sulfur compounds) with endopolygalacturonase enzyme

| Compound Name                             | Source of compound                  | Inhibition constant (mM) | ΔG (kcal mol⁻¹) | Binding site                                                                 |
|-------------------------------------------|-------------------------------------|--------------------------|-----------------|----------------------------------------------------------------------------|
| AllicinDiallylthiosulfinate                | Garlic mustard, radish, horseradish, and wasabi | 5.3                      | -3.1            | S193.I153.D191.D152.F151.V120.I127.H25.F126.H149.C150                        |
| Allyl Isothiocyanate                       | garlic                               | 5.6                      | -3              | D188.H149.I127.D152.F151.D191.D152.C150.H125.F126                          |
| (E)-Ajoen(organics)                        | garlic                               | 3.4                      | -3.3            | D124.D152.F150.C150.H149.F126.I127.H125                                  |
| 3-Vinyl-1,2-dithiin                        | garlic                               | 662                      | -4.3            | D124.D152.F150.C150.H149.F126.I127.H125.D188                            |
| Allyl Trisulfide organic                   | garlic                               | 9.9                      | -2.7            | D124.D152.F150.C150.H149.F126.I127.H125                                  |
| gamma-Glutamyl-Sallylcysteine              | garlic                               | 5.7                      | -3              | K241.T215.S237.G236.K266.D191.D152.K266.H149                             |
| Thymol                                    | thyme                                | 624                      | -4.3            | D188.H149.D152.D191.C150.F151.F19.0.V128.I127.F126.D124.H125            |
| alpha-Terpinyl acetate                    | cardamom                             | 4.1                      | -3.2            | D191.G236.K266.S237.K241.T215                                            |
| Anethole                                   | anise                                | 2.2                      | -3.6            | D81.D124.D152.F150.C150.H149.F126.I127.H125                             |
| Borneol                                    | Plant Heterotheca                    | 3.2                      | -3.4            | T215.K241.G236.S237.K266.D191                                            |
| caffeic                                   | all plants                           | 5.0                      | -3.1            | H185.N186.K266.K241.G236.T215.S237                                      |
| Carvarcol I                               | aromatic plants                     | 3.5                      | -3.3            | R264.A213.H231.D210.D209.D188.N18.6.K266                                 |
| Cinnamaldehyde                            | cinnamon trees                      | 1.6                      | -3.8            | F151.D152.F126.H149.H125.V128.C150.I127.D124                            |
| Comaric                                   | peanuts, plant.                     | 6.3                      | -3              | T215.F151.F126.C150.H125.D152.V128.H149.D191                             |
| Zingiberene                               | ginger                               | 127                      | -5.3            | D124.D152.F150.C150.H149.F126.I127.H125                                 |
| Lupenone                                  | Erica multiflora                    | 6.7                      | -7.05           | T215.F126.F125.A213.H125.H128.D152.V128..D188.S237.K266.K241            |
| Eugenol                                   | clove oil                            | 890                      | -4.1            | G236..H149.G302.Y299.R264.A213.D210.D209.D188.N186.K266.S237.K241.H149.D191.D209.D188.N186.K266.S237.K241.H149.D191.D209.D188.. |
| Limonene                                  | mint oil                             | 8.4                      | -2.8            | K266..S237.K241.H149.D191..D210.D209.D188.N186.F268.T154.H125.F151.D152.K268.D210..K266.D152.D191.H149.H125.N117.D124.S115 |
| Betulin                                   | birch bark                           | 39.0                     | -6              | D152.D191.H149.H125.N117.D124.S115                                      |
| Compound Name                          | Source of compound         | Inhibition constant mM | ΔG kcal mol⁻¹ | Binding site                                                                 |
|----------------------------------------|----------------------------|------------------------|--------------|--------------------------------------------------------------------------------|
| Betulinic Acid                         | birch bark                 | 140                    | -5.2         | G236.S237.K241.H149.D191.Y299.A213.D210.D209.D188.N186.D152.K266.K268         |
| Citric Acid organic acid               | fruits                     | 140                    | -1.1         | G236.G302.Y299.R264.K241.S237.D209.K266.N268.                                 |
| linalool                               | cannabis plant             | 5.6                    | -3           | H149.V128.F126.H149.V128.F126                                                 |
| Methylchavicol-Bornylacetat I          | Sweet Basi                 | 1.2                    | -3.9         | H149.D152.D188.N186.D191.G236.S237.K266.Y299.K241.T215.A213                  |
| Pentadecanoic acid                     | Ginger                     | 22.3                   | -2           | V128.D152.D191.K241.D191.A213.G236.S237.K266.T215                          |
| Piperine                               | Piper nigrum               | 83                     | -5.5         | V128.D152.D191.K241.D191.A213.G236.S237.T215                                 |
| Rubrenolide                            | Sextoniarubra              | 19.6                   | -2.3         | D188.H149.J127.D152.F151.D191.D152.C150.H125.F126.H231                     |
| Salcylic acid                          | Willow Bark                | 5.2                    | -3.1         | G236.N.A213.G203.Y299.R264.D210.D209.D188                                  |
| Paraphaeosphaeride A                   | Hawaiian-Plant Associated Fungus | 615                    | -4.3         | 5.G236.S237.K241.H149.D191.A213.12H.D188.F126.D152.D154.K266.             |
| 2-(1-Hydroxydodecylidene)cyclohexane-1,3-dione | Piper amalago            | 990                    | -4.1         | H149.Y299.R264.A213.D191.T215.H231.D152.C150.N186.D209.D188.N186.         |
| Methyl 2-methylsulfanyl-1H-indole-3-carboxylate | Methyl indole-3-carboxylate is the methyl ester of indole-3-carboxylic acid. It has a role as a metabolite | 2.6 | -3.5 | G236.S237.H149.Y299.R264.A213.D210.D209.D188.N186.         |
| Crassinervic Acid                      | Piper aduncum              | 83                     | -1.4         | D186.H149.D152.F126.H125.S236.S237.K266.K241                           |
Fig. 6. The overall 3D view of the modeled exopolygalacturonase enzyme when interacting with substrate in active site.

Fig. 7. Binding site resulted in hydrogen donor and acceptor for exopolygalacturonase enzyme with substrate was K314.Y349, S283.K230.K260.D255.H277.W201.D233.N231.D254.Q286.Q308.K230.L238.S258.F204
Fig. 8. Binding site resulted to hydrogen donor and acceptor for S-E-Prop-1-etyl-L-cysteine S-oxide was K314,Y349,K260,H277,W201,D233,N231,D254,D236,K230

Fig. 9. 3D structure of Sodium polygalacturonate (substrate)

Table 3. Comparative evaluation of (Inhibition constant- binding energy – Binding site) of some inhibitors in onion extract for (endopolygalacturonase enzyme)

| Compound Name                          | Source of compound | Inhibition constant mM | ΔG kcal mol⁻¹ | Binding site                                                                 |
|----------------------------------------|--------------------|------------------------|----------------|-------------------------------------------------------------------------------|
| S-E-Prop-1-etyl-L-cysteine S-oxide      | Onion plant        | 110                    | -5.4           | K241, T215, S237, G236, K266, D210, D209, N186, D188, R264, D191, H149, H231, A213, K241, T215, S237, G236, K266, D210, D209, N186, D188, R264, D191, H149, H231, Y299, A213. |
| S-3-Allylsulphinyl-L-alanine            | Onion plant        | 52                     | -5.8           | K241, T215, S237, G236, K266, D210, D209, N186, D188, R264, D191, H149, H231, Y299, A213, K241, S237, G236, K266, D210, D209, N186, D188, R264, D191, H231, Y299. |
| S-Methylcysteinesulfoxide              | Onion plant        | 104                    | -5.4           | K241, T215, S237, G236, K266, D210, D209, N186, D188, R264, D191, H231, Y299. |
Table 4. Comparative evaluation of (Inhibition constant- binding energy – Binding site) of some inhibitors in onion extract for (exopolygalacturonase enzyme)

| Compound                        | Source of compound | Inhibition constant mM | $\Delta G$ kcal mol$^{-1}$ | Binding site                                      |
|---------------------------------|--------------------|------------------------|-----------------------------|---------------------------------------------------|
| S-E-Prop-1-enyl-L-cysteine S-oxide | Onion plant        | 73                     | -5.6                        | K314, Y349, K260, H277, W201, D233, N231, D254, D236, K230 |
| S-3-Allylsulphonyl-L-alanine     | Onion plant        | 435                    | -4.5                        | K314, Y349, S283, S280, K260, D255, H277, W201, D233, N231, D254, K230, D236. |
| S-Methylcysteine sulfoxide       | Onion plant        | 305                    | -4.7                        | K314, Y349, S283, S280, K260, D255, H277, W201, D233, N231, D254. |

Fig. (10) that were obtained from the Chemoinformatics tools showed presence of carboxyl group which has (C=O 1.22 Å), (C-O 1.35 Å), (O-H 0.98 Å) bond length and carbon-hydrogen (CH$_3$,CH$_2$) has (1.096 Å) bond length in the structure of the two compounds. They have the same bond length due to binding by the same amino acids group (H149,N186,D188,D209,D210) in substrate and inhibitor of endopolygalacturonase and (K314, K230, D254, D230, N231, H277) for in substrate and inhibitor of exopolygalacturonase enzymes.

Fig. 10. 3D structure of S-E-Prop-1-enyl-L-cysteine S-oxide
Fig. 11. LC Mass Scan to S-E-Prop-1-ethyl-L-cysteine S-oxide and S-3-Allylsulphinyl-L-alanine compounds

**LC/MS scan**

Scanning of the onion extract by LC/MS confirmed the presence of active compounds where it was fragmentation to (69.9 - 87.8 - 91.1-113.8-131.9-159.8-178) Fig. (11) and (69.8-78.7-95 - 106.5-123.8 -151.9) Fig. (12) S-E-Prop-1-ethyl-L-cysteine S-oxide and S-3-Allylsulphinyl-L-alanine compounds have molecular mass M<sup>+</sup> = 178 and S-Methylcysteine sulfoxide compound has molecular mass M<sup>+</sup> =152.

**Inhibition of exopolypagalacturonaseby onion extract and kinetics study**

The enzyme assay in presence of the onion extract showed 45% inhibition percentage of the enzyme activity. The Lineweaver-Burk plot Fig. (13) showed increase in the Km value with stable Vmax value in presence of 7 µg/µL of the onion extract. The Vmax was 0.0237 and 0.022 mg/ml/min. in absence and in presence of the inhibitor, respectively; while the Km value was changed from 0.152 to 0.185 mg/ ml in absence and in presence of the inhibitor, respectively. This result indicates that the inhibitor type is competitive inhibitor.

**In-vitro evaluation of F. oxysporum inhibition by of onion extract**

Addition of 100µl of two concentrations of onion extract in the PDA showed inhibition percentage were 47% and 53% of F. oxysporum growth Fig. (14). This result is near of result of Anil and Raj 2015.
Fig. 12. LC Mass Scan to S-Methylcysteine sulfoxide compound

Fig. 13. Lineweaver-Burk plots of the exopolygalacturonase reaction in the absence and presence of the onion extract

\[ y = 8.388x + 45.39 \]
\[ y = 6.418x + 42.07 \]
CONCLUSION

Bulb Onion contains on S-E-Prop-1-enyl-L-cysteine S-oxide, S-3-Allylsulphanyl-L-alanine and S-Methylcysteinesulphoxide, compounds which have excellent binding interactions and inhibition effects on exo- and endo- polygalacturonases enzymes of Fusarium oxysporum f. sp. Lycopersici.

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تحليل معلوماتية كيميائية على بعض منظومات البكتينيز الفطري

[144]

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الموجيز

يهاجم فطر الفيوزاريم اوكربيزيك نباتات الطماطم وسببcede مراعي الدوبول الوعائي حيث يحدث العدوى بالفيوزريم من خلال انزيمات البكتينيز التي تمكن الفطر من اختراق جدار خلية العائل. تركز هذه الدراسة على استخدام برامج الكمبيوتر مثل برنامج الاوتودوك لاختيار مثبطات لإنزيمات البولي جلاكتويورينيز الداخلية والخارجية. حيث يعتمد على الامريكان جينتيك اللوريزم من خلال تقييم طاقة الارتباط وثابت التثبيط لهذه المركبات مع انزيمات البولي جلاكتويورينيز المنتجة داخل وخارج الخلايا.f

تمت هذه الدراسة تجميع هذه المركبات معمليا من ثمرة البصل الابيض جيزة 02، وتم تأكيد وجودها في مستخلص البصل بتقييمات الكروماتوجرافيا السائلة الفائقة. تم تأكيد التأثير المثبط للكلاسيكول بنيسيوم白酒يكس بمعن以其 45%، هذا بالإضافة إلى دراسة كيميائية للاستيرات. وكذلك اختبار

تأثيرها على نمو الفطر في تربة الدوبول الوعائي. وجد أن عند تركيز 20% و 40% من مستخلص البصل، حدث تثبيط لنمو الفيوزريم بنسبة 47% و 53% على التوالي. ويلاحظ ان مستخلص البصل الببتيد

له القوة على تثبيط انزيمات البكتينيز الداخلي والخارجي من خلال احتواء المستخلص على مركبات كبريتية.

الكلمات الدالة: الكيموانفورماتيكس، برنامج الاوتودوك، الفيوزريم اوكربيزيك، مستخلص البصل، الباكيتيزيوم白酒يكس، مستخلص البصل، البكتينيز جلاكتويورينيز.
تحليل معلوماتية كيميائية علي بعض مثبطات البكتينيز الفطري