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CHEMOINFORMATIC ANALYSIS OF SOME FUNGAL PECTINASES INHIBITORS

[144]

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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 exopolygalacturonase 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-Methylcysteine sulfoxide have satisfactory binding interactions and inhibition constant with endo and exopolygalacturonase. 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 exopolygalacturonase 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 exopolygalacturonase 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 endo-polygalacturonases enzymes of *Fusarium oxysporum f. sp. Lycopersici.*

Keywords: Chemoinformatic, Auto-Dock program, *Fusarium oxysporum,* Pectinases, Exopolygalacturonase; Onion extract

INTRODUCTION

Tomato (*Solanum lycopersicum L., syn. Lycopersiconesculentum* Mill.) is one of the most widespread vegetable crops worldwide. Tomato worldwide production is around115.95 million tons per year. However, the economic production of tomato crop is affected by various biotic and abiotic stress conditions **(Bergougnoux, 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 plant pathogen, a human pathogen and a toxin producer.

Pectinases are group of hydrolytic enzymes that cause pectindegradation by various mechanisms. The pectinases family includesprotopectinases, 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 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-Allylsulphinyl-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 tovisualize 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 Cygwin (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 Auto-Dock 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 $MgSO_4.7H_2O$, 0.4 g KH_2PO_4, 0.2g KCl, 1g NH_4NO_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 sulphoxides 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) desoT1lvation gas flow 900°C, source temperature 150°C and collesion 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-dinitrosaliycylic acid (DNS) method according to **Saleh et al 2009** as explained in **Table (1).**

Table 1. Exopolygalacturonases assay reagents

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 I-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-enyl-L-cysteine S-oxide, S-3-Allylsulphinyl-L-alanine and S-Methylcysteinesulfoxide. The interaction withS-3-Allylsulphinyl-L-alaninecompound has the lowestinhibition 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 I-TASSER Database search and from NCBI database. The Domain of exopolygalacturonase enzyme is Glyco_hydro _28 in range from 80 - 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 areS-3-Allylsulphinyl-L-alanine,-Methylcysteine sulfoxide and S-E-Prop-1-enyl-L-cysteine S-oxide, which showed lowest binding energy and inhibition constant **Fig. (8).**

Comparison between substrate and inhibitor

S-E-Prop-1-enyl-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-enyl-L-cysteine S-oxide

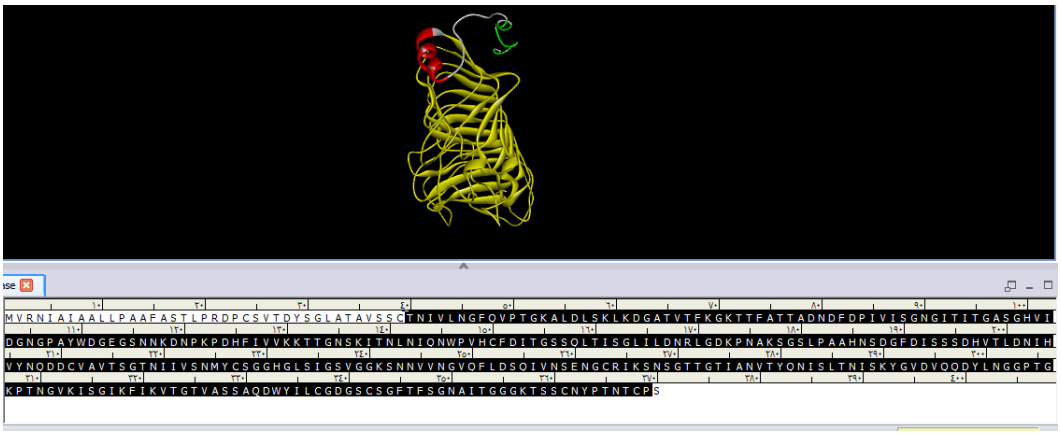

Fig. 1. 3D structure of (Glyco_hydro _28) Domain for endopolygalacturonase enzyme

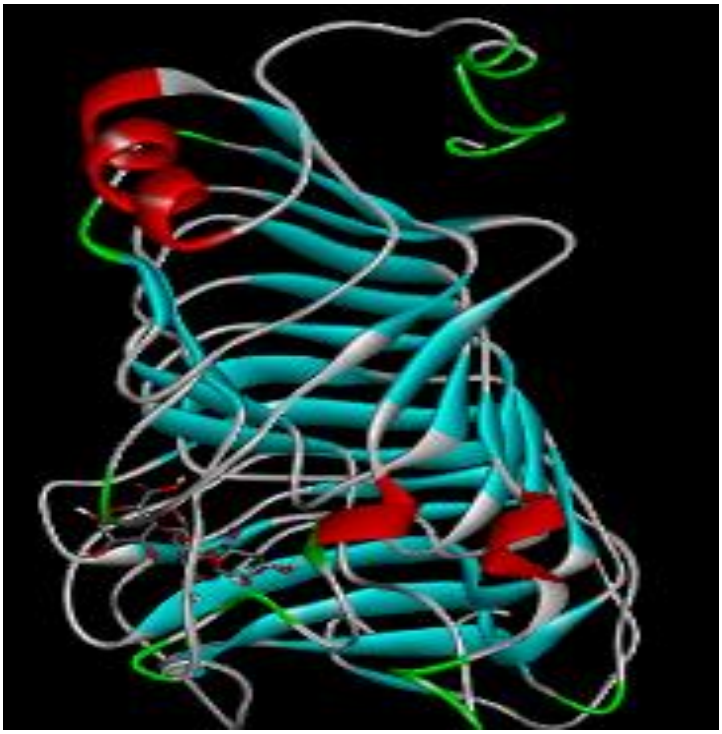

Fig. 2. The overall 3D view of endopolygalacturonase interact with substrate in the active site

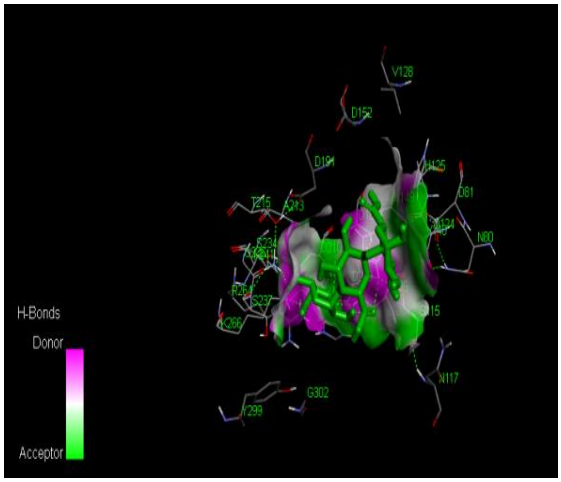

Fig. 3. The binding site resulted to hydrogen donor and acceptor for endopolygalacturonase enzyme with substrate was K241.T215.S237.G236.K266.D210.D209. N186. D188.H149.D124.N117.S115.A213.H125.N80.D81

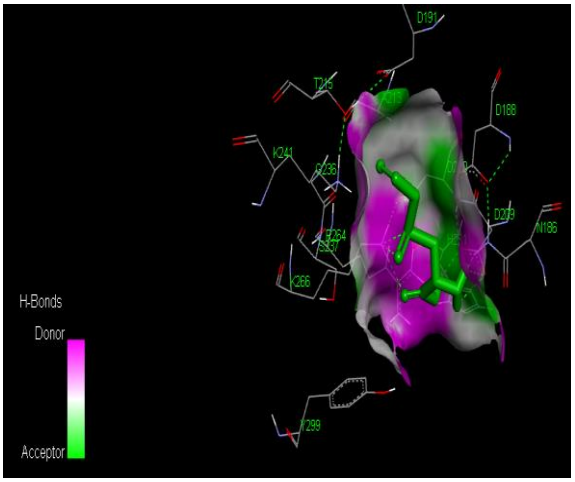

Fig. 4. .Binding site resulted to hydrogen donor and acceptor for S-3-Allylsulphinyl-L-alanine with endopolygalacturonase was K241.T215.S237.G236.K266.D210.D209.N186. D188. R264.D191. H231 .Y299.A213.

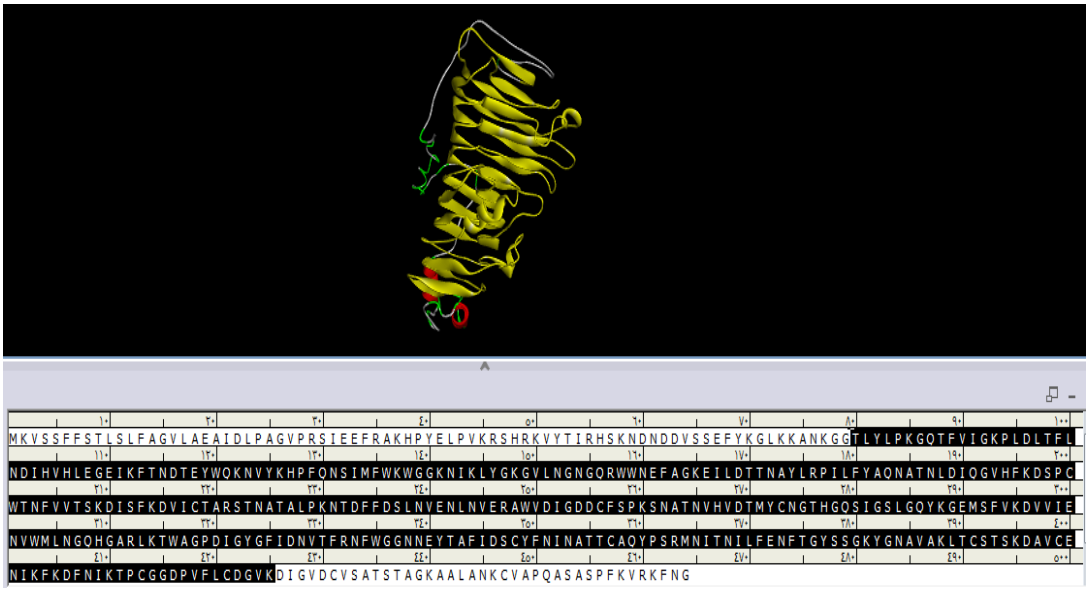

Fig. 5. 3D structure of domain (Glyco_hydro _28) for exopolygalacturonase enzyme

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
AllicinDiallylthiosul-finate	Garlic	5.3	-3.1	S193.I153.D191.D152.F151.V120.I127. H125.F126.H149.C150
Allyl Isothiocyanate	mustard, radish, horseradish, and wasabi	5.6	-3	D188.H149.I127.D152.F151.D191.D152 .C150.H125.F126
(E)-Ajoen(organic	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.H149
gamma-Glutamyl-S-allylcysteine	garlic	5.7	-3	H149.N186.D188.T115 K241.T215.S237.G236.K266.D191.D15 2.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 Heterothe-ca	3.2	-3.4	T215.K241.G236.S237.K266.D191
caffeic	all plants	5	-3.1	H185.N186.K266.K241.G236.T215.S23 7
Carvarcol l	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.C15 0.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.H125H128.D152. V128..D191.D188.S237.K266.K241
Eugenol	clove oil	890	-4.1	D124.H149.C150.F151.I127.V128.H125 ..D191
Limonene	mint oil	8.4	-2.8	G236..H149.G302.Y299.R264.A213 D210.D209.D188.N186..
Betulin	birch bark	39	-6	K266..S237.K241.H149.D191..D210.D2 09.D188.N186.F126.T154.H125.F151.D 152. K268.D210 ,K266.
Betulinaldehyde	birch bark	35	-6	D152.D191.H149H125.N117.D124. S115

Table 2. Cont.

Compound Name	Source of compound	Inhibition constant mM	ΔG kcal mol-1	Binding site
BetulinicAcid	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.F126H149.V128.F126
Methylchavicol-Bornylacetat l	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.I127.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.H231D152.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

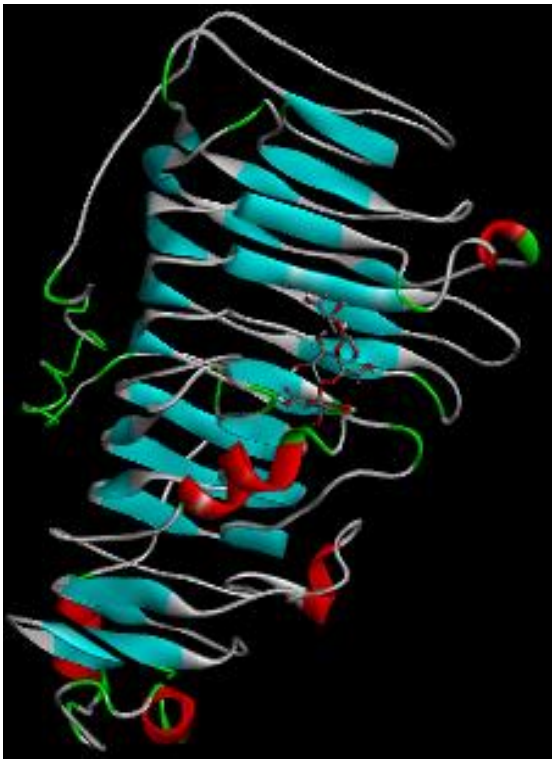

Fig. 6. The overall 3D view of the modeled exopolygalacturonase enzyme when interact with substrate in active site

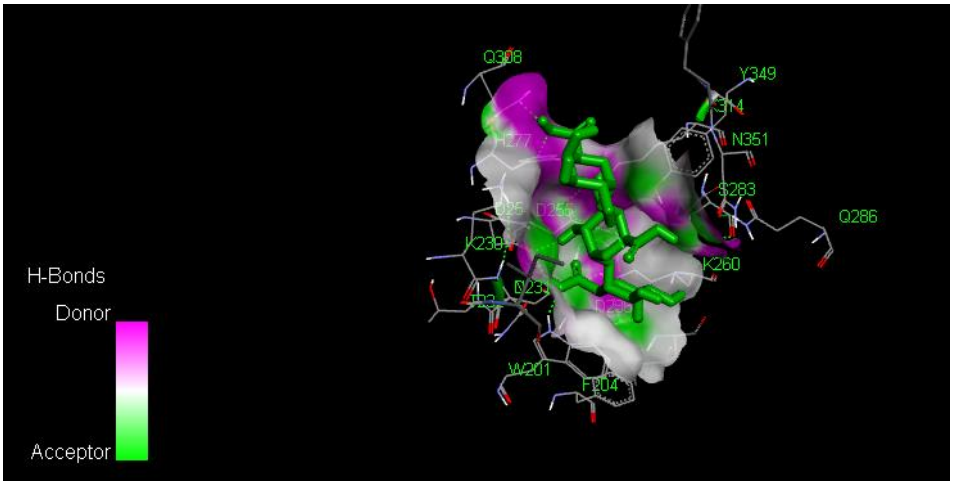

Fig. 7. Binding site resulted to hydrogen donor and acceptor for exopolygalacturonase enzyme with substrate was K314.Y349, S283.K230.K260.D255.H277.W201.D233.N231.D254.Q286.Q308.K230.L238. S258.F204

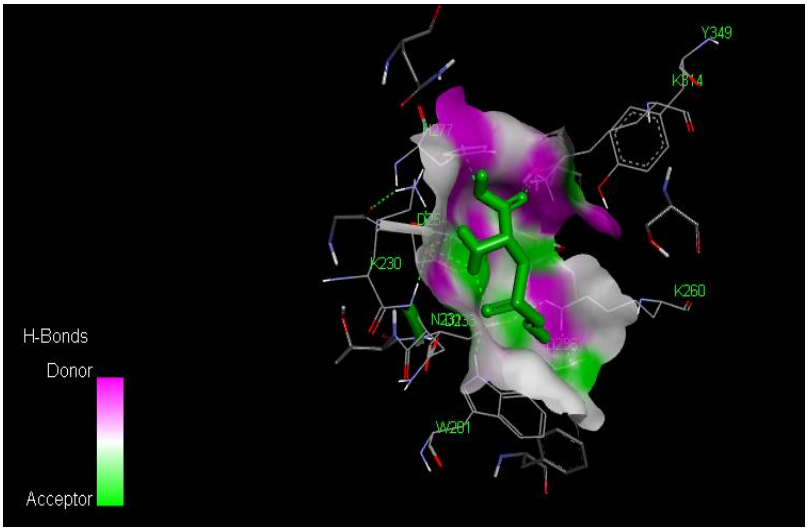

Fig. 8. Binding site resulted to hydrogen donor and acceptor for S-E-Prop-1-enyl -L-cysteine S-oxide was K314.Y349,K260.H277.W201.D233.N231.D254.D236.K230

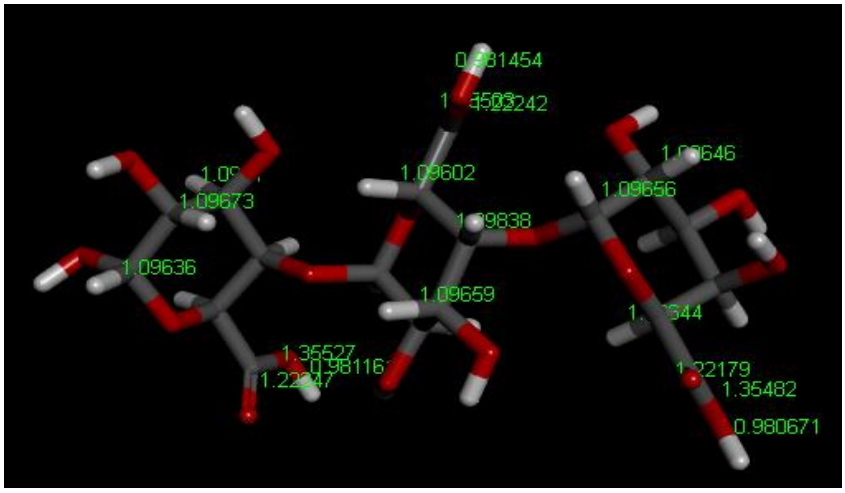

Fig. 9. 3D structure of Sodium polygalacturonate (substrate)

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-enyl-L-cysteine S-oxide,	Onion plant	110	-5.4	K241, T215, S237, G236, K266, D210, D209, N186, D188, R264, D191, H149, H231, 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.
S-Methylcysteinesulfoxide	Onion plant	104	-5.4	K241, 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	ΔG 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-Allylsulphinyl-L-alanine	Onion plant	435	-4.5	K314, Y349, S283, S280, K260, D255, H277, W201, D233, N231, D254, K230, D236.
S-Methylcysteine sulphoxide	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.

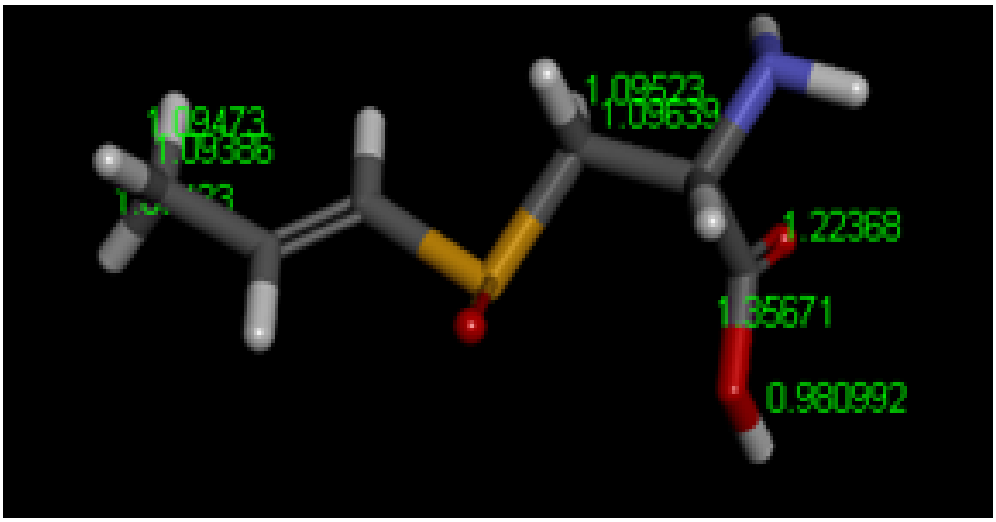

Fig. 10. 3D structure of S-E-Prop-1-enyl-L-cysteine S-oxide

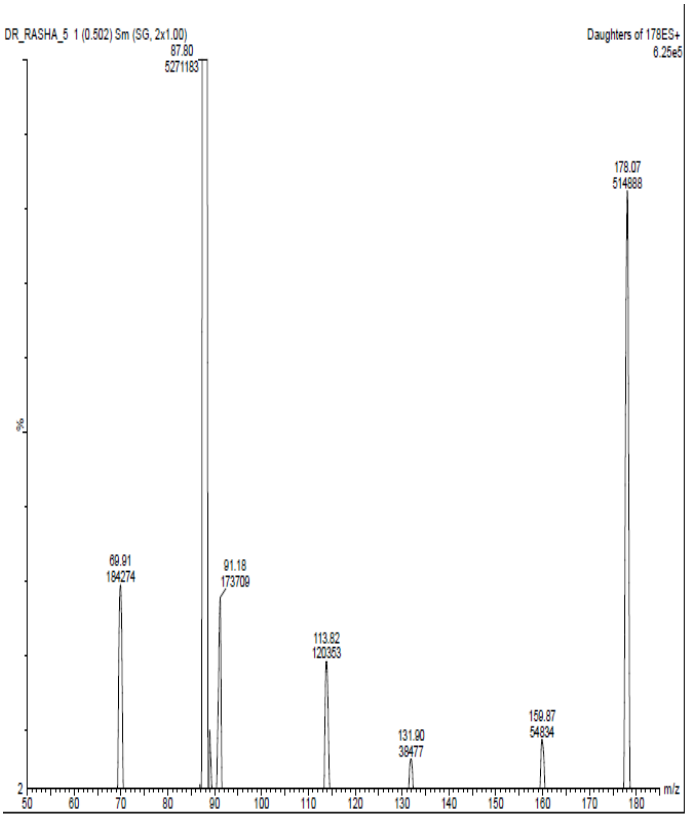

Fig. 11. LC Mass Scan to S-E-Prop-1-enyl-L-cysteine S-oxide and S-3-Allylsulphinyl-L-alanine compounds

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-enyl-L-cysteine S-oxide and S-3-Allylsulphinyl-L-alanine compounds have molecular mass M^+ = 178 and S-Methylcysteine sulfoxide compound has molecular mass M^+ =152.

Inhibition of exopolygalacturonaseby 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.**

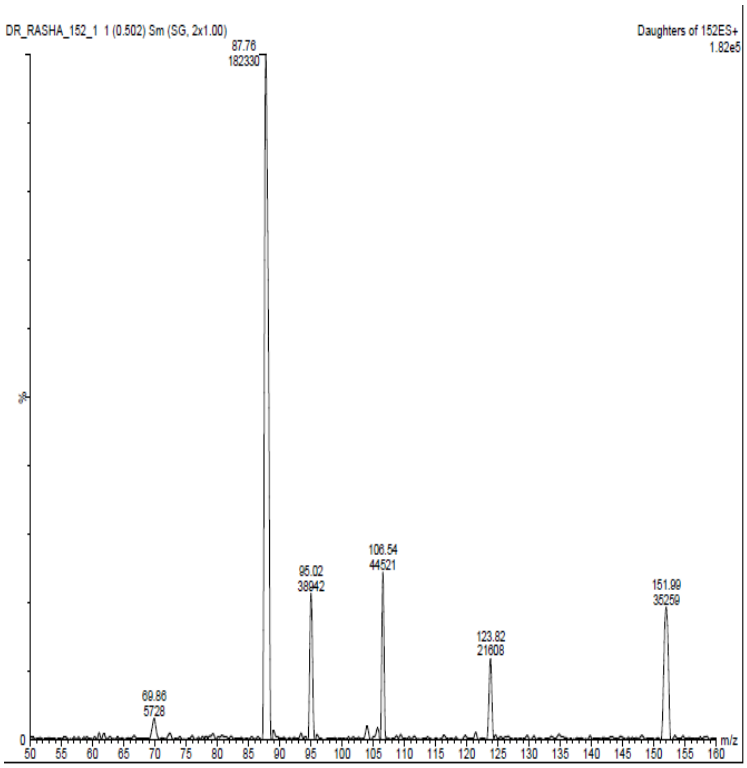

Fig. 12. LC Mass Scan to S-Methylcysteine sulfoxide compound

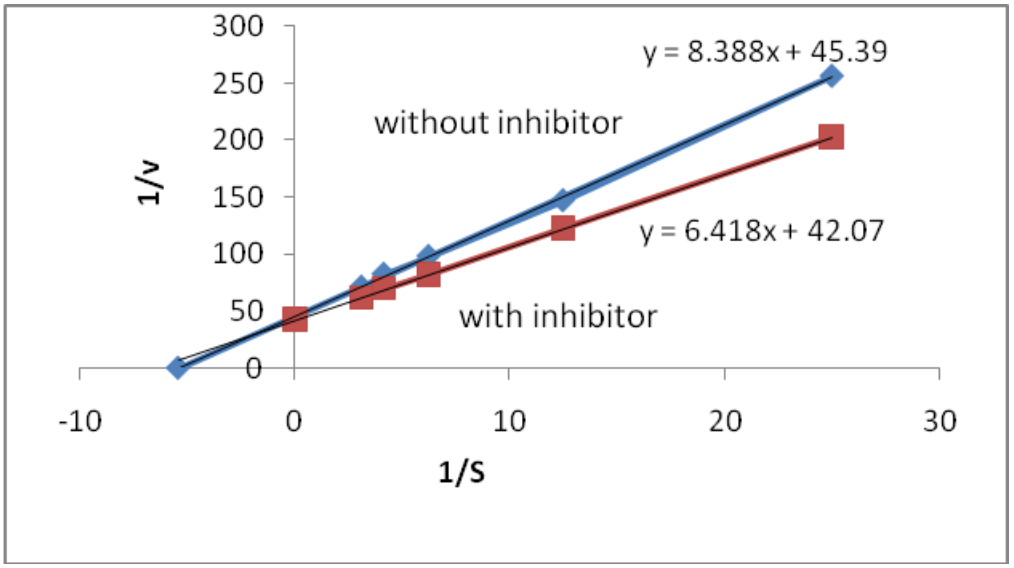

Fig. 13. Lineweaver-Burk plots of the exopolygalacturonase reaction in the absence and presence of the onion extract

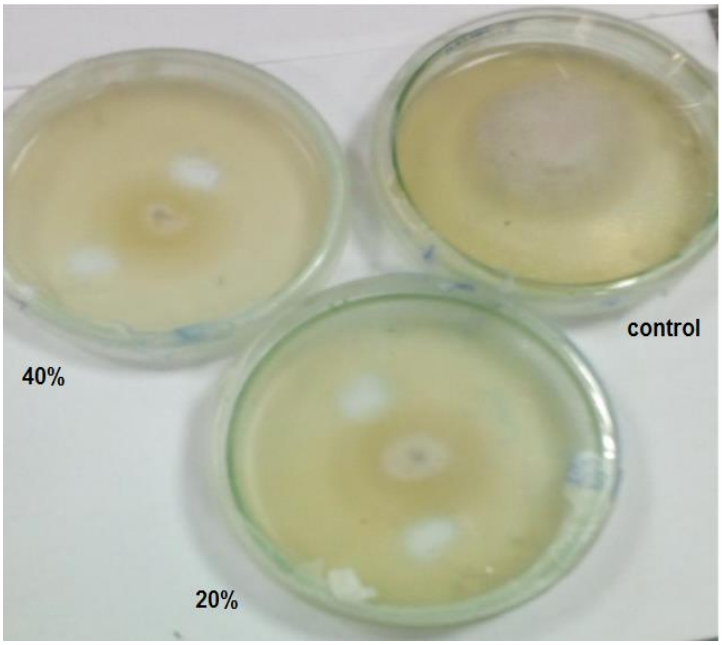

Fig. 14. Antifungal activity of onion extract inhibitors (20%, 40%).and Control

CONCLUSION

Bulb Onion contains on S-E-Prop-1-enyl-L-cysteine S-oxide, S-3-Allylsulphinyl-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 علي بعض مثبطات البكتينيز الفطري

[144]

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الموجـــــز

يهاجم فطر الفيوزاريم اوكسي سبورم ليكوبريزيك نباتات الطماطم ويسبب مرض الذبول الوعائي حيث تحدث العدوي بالفيوزريم من خلال انزيمات البكتينيز التي تمكن الفطر من اختراق جدار خلية العائل. تركز هذه الدراسة علي استخدام برامج الكمبيوتر مثل برنامج الاوتودوك لإختيار مثبطات لإنزيمات البولي جلاكتويورينيز الداخلية والخارجية . حيث يعتمد علي لامريكان جينتيك اللجوريزم من خلال تقدير طاقة الارتباط وثابت الاتزان كخصائص يتم من خلالها اختيار اقوي ارتباط لذلك تم تعريف المركبات المثبطة للبكتينيز بخصائص تشمل طاقة الارتباط واماكن تفاعلات الاحماض الامينية وثابت التثبيط مقارنة مع طاقة الارتباط وثابت التثبيط واماكن ارتباط الاحماض الامينية لهذه الانزيمات مع مادة التفاعل. انواع الاليم مثل نبات البصل لها استخدام واسع كمضادات للميكروبات والفطريات هو يحتوي في المدي بين الواحد و الخمسة في المائة وزن جاف من الاحماض الامينية الكبريتية غير البروتينية التي تعتبرمن مواد التمثيل الثانويه وتسمي مركبات اس– الكينيل-L- سيستئين سلفوكسيد وهي اس - اي-1- بروبينيل - L- سيستئين- اس - اوكسيد , اس -3- اليل سلفنيل -L- الانين, اس – مثيل سيستئين سلفوكسيد. وقد تم تحديد طاقة الارتباط واماكن تفاعلات الاحماض الامينية وثابت التثبيط لهذه المركبات مع انزيمات البولي جلاكتويورينيز المنتجة داخل وخارج خلايا الفطر . تم في هذه الدراسة إستخلاص هذه المركبات معمليا من ثمرة البصل الابيض جيزة 20, وتم تأكيد وجودها في مستخلص البصل بتحليل الكروماتوجرافي السائل فائق الكفاءة - مطياف الكتلة. تم تأكيد التاثير المثبط لهذة المركبات لانزيمات البولي جلاكتويورينيز الخارجية في المعمل بتقدير نشاط الانزيم المنتج في مزرعة الفطر في وجود وعدم وجود هذه المركبات فوجد ان مستخلص البصل الابيض يحدث تثبيط لنشاط انزيم الاكسوبولي جلاكتويورينيز بمعدل 45%، هذا بالإضافة الي دراسة كينيتكية للانزيم. وكذلك إختبار تأثيرها على نمو الفطر في تجربة الطبق البتري فوجد انه عند تركيز 20% و 40% من مستخلص البصل حدث تثبيط لنمو فطر الفيوزريم بنسبة 47% و53% علي التوالي. والخلاصة ان مستخلص البصل الابيض له القدرة علي تثبيط انزيم البكتينيز الداخلي والخارجي من خلال احتواء المستخلص علي مركبات كبريتية.

الكلمات الدالة: الكيموانفورماتيكس, برنامج الاوتودوك, الفيوزاريم اوكسي سبورم, البكتينيز, مستخلص البصل, الاكسوبولي جلاكتويورينيز

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