INT. J. BIOL. BIOTECH., 16 (3): 627-629, 2019.

ACTIVITY OF STEROIDAL ALKALOID FROM *ALLIUM VICTORIALIS* L. AGAINST UREASE AND α -GLUCOSIDASE ENZYMES

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ABSTRACT

A new compound namely allumine C (1) isolated from *Allium victorialis* L., which had been reported earlier as the novel compound studied for Urease and α-Glucosidase enzyme inhibition, as a result mild to weak inhibition was obtained for urease and α-Glucosidase with IC_{50} values 104 ± 0.61 and 108 ± 0.66 compared with standard used was Thiourea and 1-Deoxynojirimycin, respectively

Keywords: *Allium victorialis* L., steroidal alkaloid, enzymes, urease, α -glucosidase.

INTRODUCTION

Scientists are working more and more towards curing some of the critical diseases causing high fertility to the human since the inception of mankind. Biological science provided us that gateway through which various path ways become visible to cure some of the most threatening disease to human life which includes cancer, obesity, improper heart functioning etc. Among the larger class of species, Allium species are the most prominent plant species quite rich in phytonutrients, potentially provide shielding effect towards number of diseases (Barile *et al.*, 2004).

α-Glucosidase is a special type of enzyme bounded with membrane at the epithelium of the small intestine. This enzyme provides a longer time for the absorption of glucose in the blood after a meal if inhibited by using any medicinal approach. Thus, postprandial hyperglycaemia (HP) becomes lower resulting in controlling of noninsulin dependent diabetes (Anis *et al*., 2002).

Urease causes higher infectious stones in human organs leading to pathogenesis of hepatic coma and urolithiasis. It is considered as one of the most significant reasons for pathologies induced by Helicobacter pylori (HP). It gives bacteria further time to get retain inside the stomach at a favorable low pH environment eventually resultant in colonization which may produce high risk of peptic ulcer and gastric pathogenesis. To overcome these pathological problems pertinent to urease inhibition are now the primary aspect for studies and treatment of various infection diseases caused by urease-producing bacteria (Khan *et al*., 2004). In this view, a study has been reported that involves the activity of a compound Allumine C (1), obtained from the chloroform fraction of *A. victorialis* against Urease and α-Glucosidase enzymes.

METHODOLOGY

Inhibition of Urease

The main objective of this experimentation was to identify the activity of Urease by observing amount of ammonia using Indophenol method (Weatherburn, 1967). In this test of Urease inhibition, reaction mixture was formed using Jack bean urease enzymatic solution (25mm^3) and buffer solutions (55 mm^3) containing 0.1 M of urea incubated with 5mm^3 of test compound (0.0005 M) at 86^0F for 9×10^2 seconds in 96-well plates. Phenol reagent around 45$mm^3$and alkaline reagent around 70 mm^3were also added to each well (Table 1). At 630 nm a high absorbance was recorded after almost an hour on molecular device (microplate reader).

Reactions were repeated three times in a final volume of 200 mm^3. Thereafter, by using soft Max Pro software at pH 6.8, change in absorbance per minute was recorded. Percent inhibition was estimated through following equation:

% inhibition = (Ac – As)/Ac × 100

Where As = Sample absorbance and Ac = Absorbance of control

Thiourea was employed as the standard for inhibition of urease (Khan *et al.*, 2004).

The IC50 values were calculated with the help of the EZ-fit, [enzyme kinetics program (Perrella Scientific Inc., Amherst, USA)] (Khan *et al*, 2008).

Table 1. Reagents used for checking inhibition of Ureases.

Inhibition of Ureases		
Type of Reagent	Components	Conc. % w/v
Phenol Reagent	Phenol(C_6H_5OH)	1
	Sodium Nitroprusside ($C_5H_4FeN_6Na_2O_3$)	0.05
alkaline reagent	sodium hydroxide (NaOH)	0.5
	active chloride (NaOCl)	0.1

Inhibition of α-Glucosidase Assay

The method of inhibition of α-Glucosidase assay involves degeneration of substrate which results in a colored product. This effect is shown by measuring absorbance with respect to time. α-Glucosidase (Sigma, Type III, from Yeast) was first dissolved in solution of buffer A (Table 2) (Choudhary *et al*., 2011), whereas *P*-nitrophenyl-α-D-glucopyranoside was also dissolved into buffer A solution at 6mmol/L as a substrate.

Buffer B solution (102 mm^3) having 120 mm^3 sample solution, 282 mm^3 water and 200 mm^3 substrate, all these components were mixed and the resultant obtained sent inside the incubator with water bath at 98.6 ^oF for 3×10^2 seconds. After this another 200 mm^3 of enzyme based solution was added into the sample mixture.

The activity reaction of enzyme proceeded at 98.6 ^oF for 18×10^2 seconds then finally glycine buffer solution (1.2 ml) was introduced for termination of reaction.

At 410 nm absorbance was recorded to check the enzymatic activity. In this method, positive control was 1-Deoxynojiromycin hydrochloride (Ali *et al*., 2002, Matusi *et al*., 1996; Ferheen *et al*., 2009).

Table 2. Reagents used in α-Glucosidase Assay inhibition.

α-Glucosidase Assay			
Reagents	Components	Conc	pH
Buffer A solution (0.1 units/mL)	KPO_4	0.1 mol/L	6.8
	$MgCl_2$	3.2 mmol/L	
Buffer B solution (0.1 units/ mL)	KPO_4	0.5 mol/L	6.8
	$MgCl_2$	16 mmol/L	
Glycine Buffer Solution	Glycine	0.4 mol/L	10.4
Standard Sample Solution	$(CH_3)_2SO$	0.6 mg/mL	

RESULTS AND DISCUSSION

Various *Allium* species possess inhibitory activity against Urease enzyme (Olech *et al*., 2014). Quercetin glycoside from *Allium cepa* and Allicin from *Allium Sativum* were found good inhibitor against urease (Shabana *et al*., 2010; Juszkiewicz *et al*., 2004). The extracts from different species of this genus were investigated many times and revealed that some specie like *Allium fistolusum* and *Allium cepa* contain compounds quercetin and N-p-coumaroyltyramine as α-Glucosidase inhibitors but its not necessary that all species have such kind of action against this enzyme (Schmidt *et al*., 2014; Kim *et al*., 2010). The importance of this genus against inhibition of enzymes urease and α-Glucosidase prompted us to investigate the activity of newly isolated compound, Allumine C. It showed weak enzymatic activity for Urease and α-Glucosidase inhibition (Table 3).

Table 3. Urease and α-Glucosidase inhibitory effect of Allumine C.

Sample Code	Urease Inhibition IC_{50}± SEM (µM)	α-Glucosidase Inhibition IC_{50} ± SEM (µM)
Allumine C (1)	104 ± 0.61	108± 0.66
ThioUrea (Standard)	21.6± 0.03	
1-Deoxynojirimycin (Standard)		3.5± 1.70

SEM: Standard error of the mean

Allumine C (1), a steroidal alkaloid, showed urease inhibition with IC_{50} (104 ± 0.61) in comparison with thio-urea as standard resulted IC_{50} (21.6 ± 0.03). On the other hand the test of α-Glucosidase inhibition exhibited IC_{50} (108 ± 0.66) when compared with standard 1-Deoxynojirimycin revealed IC_{50} (3.5 ± 1.70). These results indicate that allumine C (1) possesses mild to weak inhibition against both enzymatic activities.

ACKNOWLDEMENT

We would like to thank Lubna Iqbal, Scientific Officer PCSIR Laboratories Karachi for help in enzymatic activity test.

REFERNCES

Ali, M. S., M. Jahangir, S. S. Hussan and M.I. Choudhary (2002). Inhibtion of α-Glucosidase by Oleanic acid and its synthetic derivatives. *Phytochemistry*, 60: 295- 299.

Anis, S., I. Anis, S. Ahmed, G. Mustafa, A. Malik, N. Afza, S.M.A. Hai, S.S. Hussan and M.I. Choudhary (2002). α-Glucosidase Inhibitory Constituents from *Cuscuta reflexa. Chem.Pharm.Bull*., 50(1): 112-114.

Barile, E., B. Zolfaghari, S.E. Sajjadi and V. Lanzotti (2004). Saponins of *Allium elburzense*. *J. Nat. Prod.*, 67: 2037-2042.

Choudhary, M.I., A. Adhikari, S. Rasheed, B.P.Marasini, N.Hussain, W.A. Kaleem and A. Rahman (2011). Cyclopeptide alkaloids of *Ziziphus oxyphylla* Edgw as novel inhibitors of α-Glucosidase enzyme and protein glycation. *Phytochemistry Letters*, 4: 404-406.

Ferheen, S., A.U. Rehman, N. Afza, A. Malik, L. Iqbal, M.A. Rasool, M.I. Ali and R.B. Tareen (2009). Galinosides A and B, bioactive flavanone glucosides from *Galinsoga Parviflora*. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 24(5): 1128-1132.

Juszkiewicz, A., Z. Anna, A. Anna and O. Zofia (2004). A study of the inhibition of jack bean urease by garlic extract. *Food Chemistry*, 85 (4): 553-558.

Khan, K.M., S. Iqbal, M.A. Lodhi, G.M. Maharvi, Z. Ullah, M.I. Choudhary, A.U. Rahman and S. Perveen (2004). Biscoumarin: new class of Urease inhibitors; economical synthesis and activity. *Bioorganic & Medicinal Chemistry*, 12: 1963-1968.

Khan, S., I. Fatima, M.H.Kazmi and A. Malik (2015). A New Steroidal Alkaloid from *Allium victorialis. Chem of Nat Comp.*, 51 (6): 1134-1137.

Khan, S., Y.Shazia, A. Nighat, M. Abdul, I. Lubna and L. Mehreen (2008). Cotonoates A and B, New Aromatic Esters from *Cotoneaster racemiflora. Z. Naturforsch*, 63b: 1219-1222.

Kim M.H., H.J. Sung, J. Hae-D. S.L. Mee and K. Young-I (2010). Antioxidant Activity and α-Glucosidase Inhibitory Potential of Onion (Allium cepa L.) Extracts, *Food Sci. BioTechnol.*, 19 (1): 159-164.

Matsui, T., C. Yoshimoto, K. Osajima, T. Oki and Y. Osajima (1996). *In Vitro* Survey of α-Glucosidase Inhibitory Food Components. *Biosci. Biotech. Biochem*, 60: 2019- 2022.

Olech, Z., W. Zaborska and M. Kot (2014). Jack bean urease inhibition by crude juices of *Allium* and *Brassica* plants. Determination of thiosulfinates, *Food Chemistry*, 145:154-160.

Schmidt, J.S., T.N. Nil and S. Dan (2014). Assessment of constituents in *Allium* by multivariate data analysis, high-resolution α-glucosidase inhibition assay and HPLC-SPE-NMR. *Food Chemistry*, 161:192-198.

Shabana, S., K. Azusa, K. Kenji, A. Kohki and Hideo (2010). Inhibitory Activity against Urease of Quercetin Glycosides Isolated from Allium cepa and *Psidium guajava. Bioscience, Biotech. & Biochemistry*, 74 (4): 878-880.

Weatherburn, M. W. (1967). Phenol-Hypochlorite Reaction for Determination of Ammonia. *Anal. Chem*., 39: 971.

(*Accepted for publication June 2019*)