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Evaluation the Antioxidant Activity Of Syrian Ficus And Olive Leaf Extracts And their Inhibitory Effects On α -Glucosidase In Vitro.

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Abstract

In this work we examined the effectiveness of ethanolic and aqueous extracts of Syrian ficus leaf and their mixes with Syrian olive leaf extracts (1:1, 5:1, 7:1) (olive: ficus) in inhibition of both of 2,2-diphenyl-1 picrylhydrazyl ($DPPH^{\cdot}$), 2.2,-azino-bis-[3-ethyl benzothiazoline-6-Sulfonic Acid] ($ABTS^{+\cdot}$). The study also investigated the inhibition of both of ficus and olive leaf extracts and their mixe (1:1) on α-glucosidase in order to investigate the ability of these extracts to reduce blood sugar in vitro. The results showed that there were significant differences in the ability of the mixes to inhibit $DPPH^{\cdot}$, the lower IC_{50} value was 0.24±0.01 mg/ml for the mix (1:1), as oppose to the results with the $ABTS^{+\cdot}$, there is no significant differences in the ability of the mixes (1:1, 5:1) and ethanolic olive leaf extract to inhibit $ABTS^{+\cdot}$, (IC_{50}=0.47±0.01 mg/ml). The Inhibition percentage of α-glucosidase reached 98.5% at 7.69 mg/ml and 81.34% at 3.85 mg/ml for aqueous ficus and ethanolic olive leaf extracts, respectively. The ethanolic olive leaf extract gave the lower value (IC_{50} = 0.34±0.12 mg/ml). The α-glucosidase inhibition mode was also tested, the max velocity V_{max} and Micheles-Menten constant K_m were calculated from the Lineweaver-Burk equation. The result showed that inhibition modes were non-competitive and competitive types for ficus and olive leaf extracts, respectively.

Keywords: *Syrian ficus and olive leaf, Antioxidant activity, α- glucosidase activity inhibition, inhibition Mode*

1. Introduction

Diabetes mellitus is one of the most interrelated and universal public problems. Its responsible for about 5 percent of global deaths[1]. The number of people suffering from this disease worldwide is increasing at an alarming rate with an predicted 600 million people likely to be diabetic by the year 2035 as against 191 million estimated in 2000 [2,3]. The chronic hyperglycemia is the underlying symptom which eventually culminates into abnormal carbohydrates, fat and protein metabolism due to disorders in insulin secretion or action. The most common is type tow caused by a decreased sensitivity of target cells to insulin[4]. One curative way for treating diabetes is to decrease the postprandial hyperglycemia, through retarding the absorption of glucose by the inhibition of the carbohydrate hydrolyzing enzymes, as α-glucosidase in the digestive tract[5]. Inhibitors of α-glucosidase decrease the postprandial blood glucose excursion levels in diabetic patients and delay the breakdown of carbohydrate in the small intestine[6]. The inhibition of this prominent enzyme has been found as effective and useful strategy to lower the levels of postprandial hyperglycemia[7]. There are some anti diabetics medical drugs which act by inhibiting α-glucosidase activity, namely acarbose, voglibose and miglitol[8], but the constant use of these drugs is often linked with undesirable side effects, such as adverse gastrointestinal symptoms and liver toxicity [9,10]. For this reason there is a need for natural α-glucosidase inhibitors which have unwanted secondary or minimal effects. Plants play an important role in the treatment of diabetes, as cost effective alternative to modern drugs. Different plant parts were used in the treatment of diabetes the most frequently was the leaf (35%) followed by seeds (20%), fruits (15%) and rhizomes (10%)[11]. Syrian Arab Republic is a richness and diversity country, which is attributed to its topographical and climatic diversity. Ficus belongs to the Moraceae and there are at least 800 species of ficus around the world. The total cultivated area of ficus in Syrian Arab Republic is 9663 ha, with a total production of $35.7*10^3$ tons [12,13]. Ficus leaf tea is a popular remedy for diabetes, due to its richness in bioactive compounds, their extracts contain the highest percentage of total phenols and flavonoids which are antioxidants[14]. Rutin flavonoids were found to be the major flavonoids in ficus leaf, as well as quercetin and luteolin[15]. Many Studies have shown the effectiveness of ficus leaf extract in inhibiting both alpha-amylase and α-glucosidase enzymes as they prolong total carbohydrate digestion time, resulting in low blood glucose uptake [16,17]. Another study was conducted on rats with diabetes, and their blood glucose level was controlled by giving them ficus leaf extract for four-days[18]. Olive leaf tea is also a common folk remedy for diabetes. Studies have shown the effectiveness of olive leaf extract in inhibiting pancreatic amylase[19], which extends the total digestion time of carbohydrates, resulting in low glucose uptake[16]. In another study, the ability of olive leaf to reduce high blood glucose levels after starch intake for volunteers, and thus may be a useful dietary supplement for the treatment of diabetes[19]. Oleuropein (the most abundant compound in olive leaf) has an anti hyperglycemic effect in diabetic rats [20,21]. The concentration of oleuropein varies according to the environment and climate[22]. Syrian olive leaf contain a high concentration of 88.50 ± 9.6 mg / g in ethanolic extracts[23].

Therefore, the aim of this study was to exploit the local environment plants by investigating the antioxidant activities of Syrian ficus and olive leaf extracts and their synergistic interaction as mixes in order to scavenge free radicals and to inhibit α-glucosidase potentials as a possible mechanism behind the hypoglycemic action of these plants and their usage in the management of diabetes mellitus as a available cheap natural source. as well the determination of modes of inhibition of this enzyme.

2. Materials and methods

2.1. Experimental section

2.1.1. Plant material

Ficus leaf were collected on April from Banias (Latakia- Governorate), Syria. Olive leaf were collected in June from

khan Arnabeh (Quneitra Governorate), Syria. The plants were identified by Prof. Jurjet Babojian (Department of Plant Biology, Faculty of science, Damascus University, Syria). The leaf were dried in the shade away from the sunlight for 10 days, ground with an electric mill, and then stored in a dark, dry, tightly sealed place at room temperature until use.

2.1.2. Chemicals and reagents

Rutin Hydrate standard 95%, oleuropein standard 98.0%, α-glucosidase from *Saccharomyces cerevisiae* and 4-nitrophenyl α-D-glucopyranoside (Pnpg) were products of Sigma-Adrich Co, USA. Other chemicals and reagents used were of analytical grade

2.1.3. Instruments

Vortex MS1 Mini shaker (KAI), ultrasonic water bath Model Trans sonic 460/H (Elma). Rotary evaporator, UV-VIS spectrometer (JASCO- Japan).

2.1.4. Preparation of extracts

1g of both of studied leaf powders was added to 20 mL ethanol 70% for ethanolic extracts, stir well by piping for homogenization for one minute, then placed in the ultrasonic bath at 75°C, for 30 minutes, filtered with (0.45µm) filters. Repeat the extraction 3 times. The aqueous extracts were prepared in the same way with 20 mL distilled water. The ultrasonic extraction method was used for its ability to improve extraction by speeding up the release of bioactive substances from cell walls and facilitating their ease of transference[24]. The extracts were evaporated by the rotary evaporator until dry, then dissolved with dimethyl sulfoxide to study its efficacy in inhibiting the α-glucosidase.

2.1.5. Anti oxidant Activity

2.1.5.1. DPPH$^{\cdot}$ Radical Scavenging activity assay

The effect of both of the ficus and mixes extracts on DPPH$^{\cdot}$ was estimated according to the literature[25]. The effect of olive leaf extracts was studied in previous work[23] but mentioned for comparison. 3 ml of freshly prepared ethanolic DPPH$^{\cdot}$ solution (45 µg/ml) was mixed with 300 µl of the extract samples at varying concentrations range. The mixture was shaken vigorously and allowed standing for 30 minutes at room temperature in the dark. The absorbance was measured at 515 nm with a spectrophotometer. The percentage inhibition was calculated as Equation (1) Where: A_b is the absorbance of the control reaction, and A_a is the absorbance of the sample. The IC_{50} values were calculated by the linear regression method of plots of the percent of antiradical activity against the concentration of the tested compounds:

$$\mathbf{I}_{\mathbf{DPPH}^{\cdot}}\ \mathbf{\%} = [(A_b - A_a)/A_b] \times 100 \quad (1)$$

2.1.5.2. ABTS$^{+\cdot}$ radical scavenging activity assay:

The total antioxidant activity by (ABTS$^{+\cdot}$) assay was determined according to the method of [26] with little modifications. The effect of olive leaf extracts was studied in previous work [23] but mentioned for comparison. The ABTS$^{+\cdot}$ cation radical solution was prepared by reacting similar quantities of 7mM of ABTS and 2.45 mM of sodium persulphate solutions for 16 hours at 3^{o}C in the dark. Before using this solution, it was diluted with distilled water to obtain an absorbance of (0.75 ± 0.02) at 734 nm. The reaction mixtures composed of 3ml of ABTS$^{+\cdot}$ solution and 200 µl of extracts at different concentrations range. The absorbance was measured at 734 nm by using a spectrophotometer. The percentage inhibition was calculated as Equation (2) Where A_b is the absorbance of the blank

and A_a is the absorbance in the presence of extract. The IC_{50} values of extracts, oleuropein standard, and the mixes (1:1, 5:1, 7:1) were calculated by the linear regression method of plots of the percent of antiradical activity against the concentration of the tested compounds and compared with that of ascorbic acid.

$$I_{ABTS^{+\cdot}}\ \% = [(A_b - A_a)/A_b] \times 100 \quad (2)$$

2.1.6. In vitro assay for α-glucosidase

The effect of both of ficus and olive extracts on α-glucosidase activity was determined according to the method described by Kim *et al*. [27] with little modifications. The substrate solution p-nitropheynyl glucopyranoside (pNPG) (3.0 mM) was prepared in 0.02 M phosphate buffer, pH 6.9. 100 µL of α-glucosidase (0.1U/ml) was pre-incubated at room temperature with 100 µL of the different concentrations of the extracts (ethanolic and aqueous) for 10 min. Then 50 µL of 3.0 mM (pNPG) as a substrate dissolved in 0.02M phosphate buffer (pH 6.9) was added to start the reaction. The reaction mixture was incubated at 37°C for 20 min and stopped by adding 1250 µL of 0.1 M Na_2CO_3. The α-glucosidase activity was determined at 405 nm by measuring the yellow colored para-nitrophenol released from pNPG. The results (% Inhibition) are expressed in Eq 3.

% Inhibition = [(Acontrol-Aextracts)/Acontrol] x 100. (3)

where Acontrol and Aextracts are the absorbance of the control and extract, respectively. The IC_{50} values were calculated by the linear regression method of plots of the percent of antiradical activity against the concentration of the tested compounds.

2.1.7. Mode of α-glucosidase inhibition

The mode of inhibition of the ficus and olive leaf extracts was determined using the extracts with the lowest IC_{50} according to the method described by Ali *et al* [5] with some modification. Briefly, 100 µL of the extract was pre-incubated with 100 µL of α-glucosidase solution for 10 mins at 25^{0}C in one set of tubes. In another set of tubes α-glucosidase was pre-incubated with 100 µL of phosphate buffer (pH 6.9). 50 µL of PNPG at increasing concentrations was added to both sets of reaction mixtures to start the reaction. The mixtures were then incubated for 10 mins at 37°C, and 1250 µL of Na_2CO_3 was added to stop the reaction. The amount of reducing sugars released was determined by spectrophotometer measurement using a pNPG standard curve and converted the concentration to reaction velocities. A double reciprocal plot (1/v versus 1/[S]) was plotted, where v is reaction velocity and [S] is substrate concentration. The type (mode) of inhibition of the extracts on α-glucosidase activity was determined by analysis of the double reciprocal (Lineweaver-Burk) plot using Michaelis-Menten kinetics Eq 4.

$$1/V = (K_m + [S]) / V_{max}[S] = K_m/V_{max}[S] + 1/V_{max} \quad (4)$$

2.2. Statistical analysis

For statistical analysis, Statistical Package for the Social Science (SPSS, 20) was used. Data were expressed as mean ± SD of three different experiences. Comparisons for IC_{50} values were performed by One-way ANOVA analysis of variance, with post hoc test (Tukey,LSD tests), the significance level was P <0.05.

3. Results and Discussions

3.1. Scavenging activity of DPPH· and ABTS$^{+\cdot}$ radicals

The scavenge effects of olive leaf extracts on DPPH· and $ABTS^{+\cdot}$ radicals were investigated in previous work [23]. In this work we attempt to investigate the Synergic mixture effect of both of ficus and olive leaf extracts in different

ratios (1:1, 5:1, 7:1) in Scavenging activity of both of DPPH· and $ABTS^{+\cdot}$ radicals. The estimation of the antiradical capacity of the different extracts was performed by determining the value of IC_{50}, Figure 1. shows for DPPH· test, the scavenge activity of the mixes extracts (1:1, 7:1) were more effective than the ethanolic olive and the ethanolic ficus extracts separately. According to the statically studies, there is significant differences in IC_{50} values for them all except the mix (5:1). The mix (1:1) gives the lowest IC_{50} value followed by the mix (7:1) then ethanolic olive leaf extract. This indicates that mix (1:1) is more effective than each extract separately in Scavenging activity of DPPH· Radicals. There is no significant differences in IC_{50} values for both of mixes (1:1) and (5:1) and ethanolic olive leaf extract in the $ABTS^{+\cdot}$ Test, Figure 2. They give lower IC_{50} value than ficus leaf extracts. This indicates that olive leaf extracts are more effective in Scavenging activity of $ABTS^{+\cdot}$ Radicals.

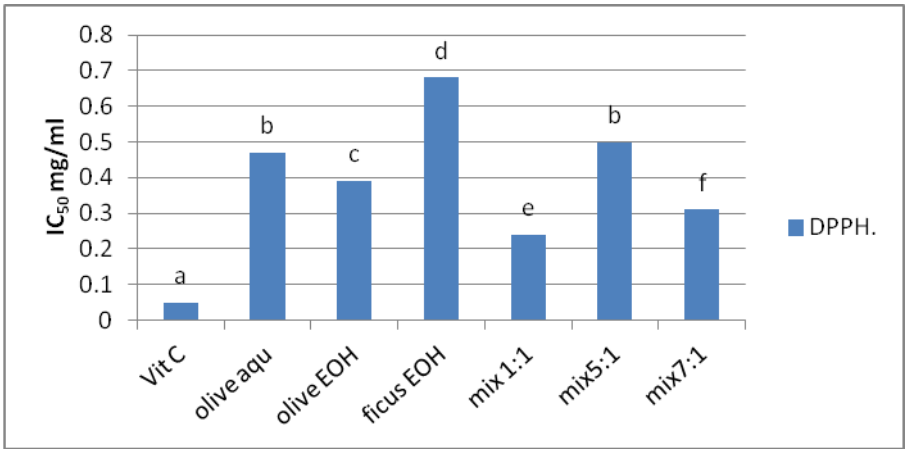

Figure 1. The Values of IC_{50} on DPPH· Assay, a,b,c,d,e,f Values are differ significantly from each other according to one way ANOVA(Tukey test, $P<0.05$).

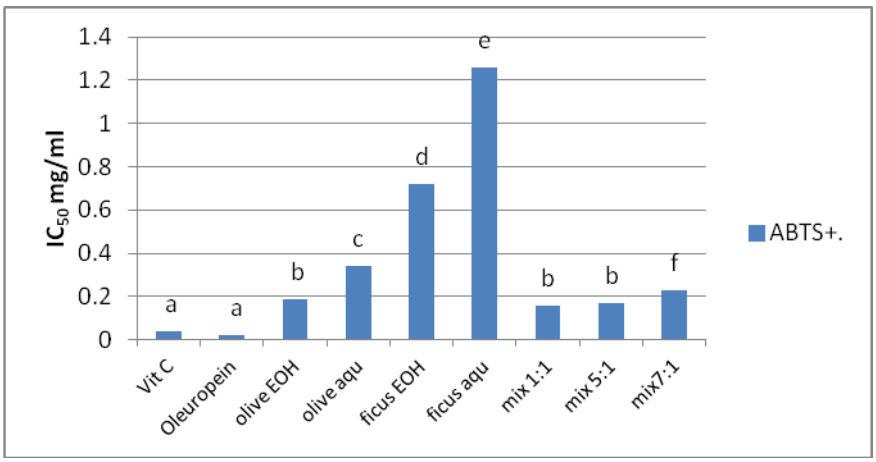

Figure 2. The Values of IC50 on $ABTS^{+\cdot}$ Assay, a,b,c,d,e,f Values are differ significantly from each other according to one way ANOVA (Tukey test, $P<0.05$).

3.2. α-glucosidase inhibition

The inhibitory effect of both of aqueous, ethanolic leaf extracts was studied using different concentrations. It was observed that the aqueous extracts gave higher inhibition values than ethanolic extracts in ficus leaf extract, the percentage of inhibition was 98.51% at a concentration of 7.69 mg/ml. While the ethanolic extract of the olive leaf gave the highest efficacy of inhibition compared with the aqueous one, the percentage of inhibition was 81.34% at a concentration of 3.85 mg/ml. Figure 3,4. This may due to the nature of the compounds that extracted from studied leaf.

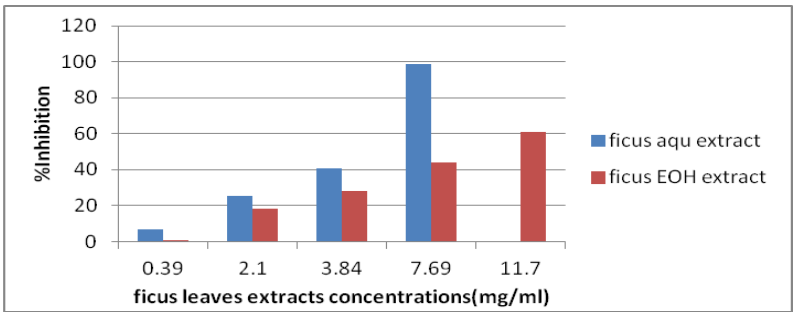

Figure 3. Percentage inhibition of α-glucosidase by different extracts of ficus leaf

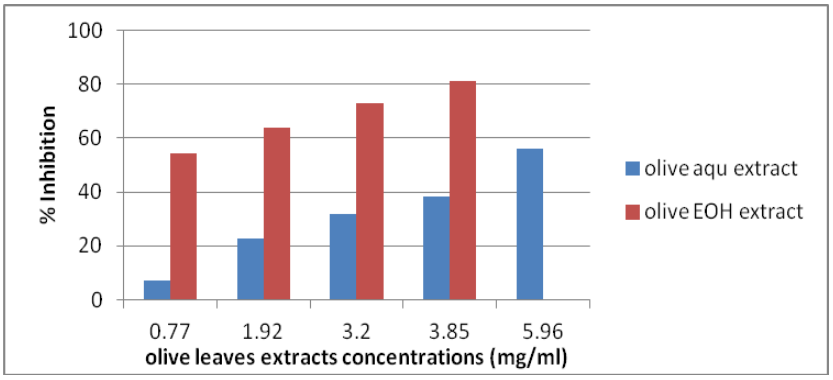

Figure 4. Percentage inhibition of α-glucosidase by different extracts of olive leaf

The IC_{50} values were calculated for aqueous and ethanolic leaf extracts, mix (1:1) , rutin and oleuropein standards. The mix (1:1) extract has a lower value of IC_{50} as shown in Table 1. But there is no significant differences between mix (1:1) and both of ethanolic olive leaf extract and oleuropein standard according to statical study.

Table 1: IC_{50} values of α-glucosidase inhibition by leaf extracts of the studied plants

Leaf	extracts	IC_{50}(mg/ml)
Ficus	Aqueous	3.86 ± 0.57^{a}
	Ethanolic	9.12 ± 0.14^{b}
Olive	Aqueous	5.21 ± 0.20^{c}
	Ethanolic	0.34 ± 0.12^{d}
Mix	1:1	0.13 ± 0.04^{d}
Rutin	std	1.01 ± 0.01^{e}
Oleuropein	std	0.27 ± 0.01^{d}

a,b,c,d,e Values are differ significantly from each other according one way ANOVA ($P<0.05$),

3.3.Mode of α-glucosidase inhibition

The Lineweaver-Burke plot was generated to determine the mode of inhibition of the enzyme, Figure 5 shows decreasing in the maximal velocity value (V_{max}), and the Michaelis-Menten constant (K_m) value nearly constant. This means that the aqueous ficus leaf extract acted as a non-competitive inhibitor of α-glucosidase. Non-competitive inhibitors do not compete with the substrate to bind the active region of the free enzyme they bind to enzyme-substrate complexes, resulting in enzyme-substrate inhibitor complexes [28]. Figure 6 shows the Lineweaver-Burke plot for ethanolic olive leaf extract with constant maximal velocity value (V_{max}), and increasing in Michaelis-Menten constant

(K_m) value, thus a near competitive mode of inhibition of the α-glucosidase. This implies the active components of the ethanolic olive leaf extract compete with the substrate for binding to the active site of the enzyme, thereby preventing or slowing down the breakdown of oligosaccharides to disaccharides, that means the ethanolic olive leaf extract acted as a competitive inhibitor of alpha-glucosidase [29]. The responsibility for this action is due to the major compounds rutin and oleuropein respectively in both of these extracts as shown in Figure 7,8.

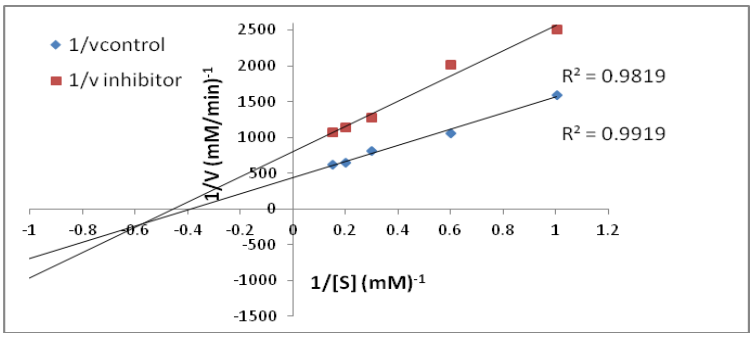

Figure 5. Mode of inhibition of α-glucosidase by aqueous extract of ficus leaf

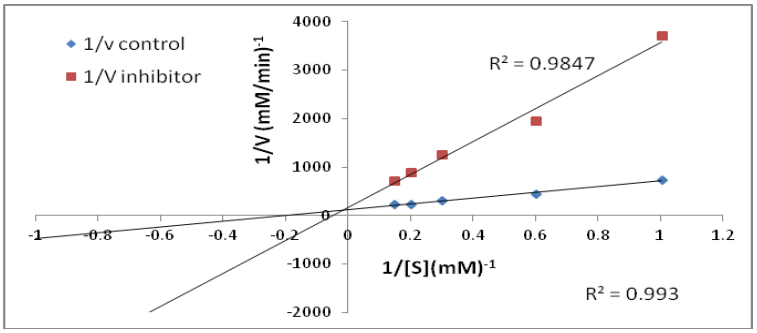

Figure 6. Mode of inhibition of α-glucosidase by ethanolic extract of olive leaf

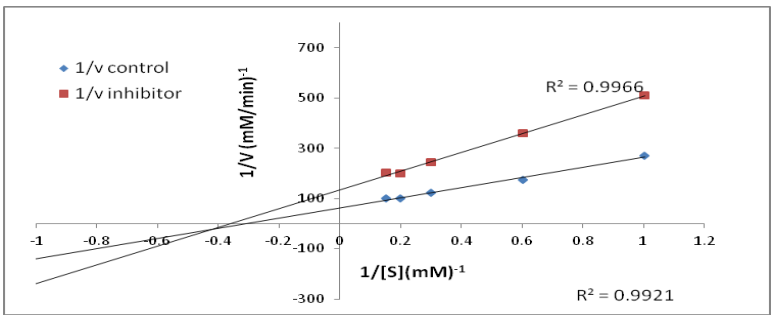

Figure 7. Mode of inhibition of α-glucosidase by rutin standard

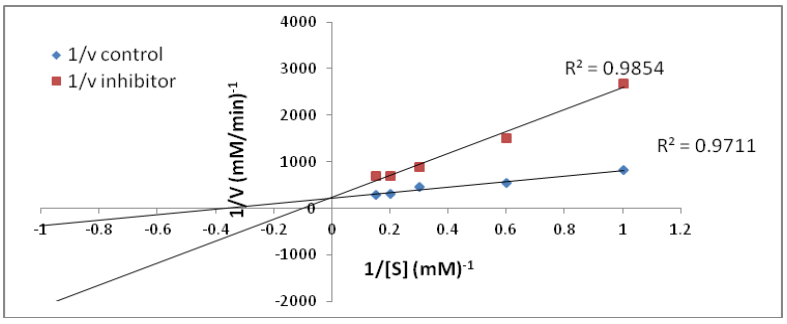

Figure 8. Mode of inhibition of α-glucosidase by oleuropein standard

4. Conclusion

This study reports that both of Syrian ficus and olive leaf extracts are good natural antioxidant source. But the ethanolic one is more effective than the aqueous one. The mix of two leaf extracts (1:1) is more effective than each extract separately in Scavenging activity of $DPPH^{\cdot}$ radicals, but the olive leaf extracts are more effective in Scavenging activity of $ABTS^{+\cdot}$ radicals. The mix (1:1) extract has the best inhibiting a-glucosidase activity comparing with the other extracts. The aqueous ficus leaf extract acted as a non-competitive inhibitor of α-glucosidase, but the ethanolic olive leaf extract acted as a near competitive mode of inhibition of the α-glucosidase, thereby preventing or slowing down the breakdown of oligosaccharides to disaccharides, this open ways for using ficus and olive leaf as a cheap and natural anti diabetic treatment.

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