In-vitro and in-silico strategies to assess the unexplored pharmacological lineages of Solanum elaeagnifolium Cav. Leaves

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Abstract

The current work aims to investigate the chemical components in *Solanum elaeagnifolium* leaf extracts and their pharmacological potential in antioxidant, antibacterial and anti-diabetic effects. To screen the complete antioxidant potential, the DPPH, SO, FRAP, MCA, and PHM assays were evaluated; whereas the antibacterial activity was determined by the minimum inhibitory concentration method against 10 pathogenic microorganisms. Also, the α-amylase and α-glucosidase effects were explored to know the enzyme inhibitory potentials of this plant leaves. After obtaining possible drug probabilities in the crude extract exploration, the GC-MS screened molecules were docked with diabetes-related proteins to screen for active anti-diabetics. Ultimately, as a final result of this exploration, the following values are lodged for future reference for those wishing to work on this plant. Firstly, the quantitative phytochemicals exploration revealed that the ethyl acetate extract has the highest content of TPC with 79.04 ± 0.98mg/g GAE and TFC with 79.04 ± 0.98mg/g GAE and 134.31 ± 0.04 mg/g QE. Similarly, the tested extracts in this study showed significant antioxidant activity at various concentrations; whereas the extracts used against the bacteria showed 50% inhibition against the pathogens tested.The ethanol extracts were subjected to an MIC according to the bacteriostatic potential; the concentration of 118.75 g/mL showed remarkable inhibition potentials against *E. coli, Proteus vulgaris, Staphylococcus epidermis, Bacillus subtilis* and *Rhodococcus equi*. In the enzymatic exploration of α-amylase and α-glucosidase, IC50 values of ethanol extracts were found to be17.78 ± 2.38µg/ml and 27.90 ± 5.02 µg/ml followed by acetone (17.96 ± 6.05µg/ml and 36.44 ± 3.30 µg/ml). Through *in-silico* exploration, we found that *S. elegans* phytochemicals have potential drug-binding affinities and better docking scores with diabetic proteins compared to the corresponding drugs. Based on the various pharmacological investigations, the present study concluded that *Solanumelaeagnifolium* might have possible drug candidates as antioxidant, antibacterial and antidiabetic agents. As a result of the present study, we conclude that the phytochemicals may have revealed their therapeutic potential either alone or synergistically with other phytochemicals of this leaves.

1. Introduction

Solanumelaeagnifolium is considered a weed plant in Greece, India, Israel, Zimbabwe, Sicily, South Africa, Spain and some other countries [1]. This plant belongs to the members of the nightshade family (Solanaceae).Solanum is the most widespread genus of the nightshade family worldwide, consisting of 1500 species [2]. It is a deeply rooted perennial that causes adverse effects on agricultural crops, affecting 70% of yields in economically important crops by harboring pests and diseases [3]. *S. elaeagnifolium* is considered an indomitable weed, particularly in many arid to semi-arid regions [4]. Besides, it competes with a variety of plants for moisture and plant nutrients in both arid and irrigated environments; where it infiltrates habitats and dominates native species by forming dense distribution [1]. Besides vegetative reproduction through rhizome and root fragments, is also reproduces sexually through seeds.

In general, Solanum species are reported to be rich in steroidal alkaloids Solasodine. Specifically, the berries of *S. elaeagnifolium* possess this phytochemical [6]. In addition to this phytochemical, the leaves and seeds of this plant have been found to have a wide range of polyphenolic molecules, including β-sitosterol-3-O-β–D-glucoside, Quercetin-3-O-β-D-glucopyranoside, β-sitosterol, stigmasterol, kaempferol, Cglycoside, kaempferol 8-C-β-galactoside, 2-(2-hydroxyphenoxy)-3,6,8-trihydroxy-4H-chromen-4-one, Quercetin, Rutin, Mangiferin, 2R, 3R-

5,7, 4'-trihydroxy-dihydroflavon-3-O-α-D-glucopyranosyl 6"-O-β-dglucopyranoside-6"'-p-hydroxy benzoate, kaempferol-3-6'- coumaroylglucoside, chlorogenic acid, kaempferol β-D-(6”-O-cis-cinnamoyl glucoside), coumaroylglucoside, mangiferin, kaempferol, coumaroyl quince acid and dicaffeoylquinicacids [5]

Although the plant consists of such pharmacologically active molecules, *S. elaeagnifolium* seeds and leaves are also reported to have potent insecticidal and repellent potential against plant pests; therefore, it is supplied as an alternative pesticide for agricultural crops [1] On the other hand, Solanine, a unique phytochemical found in Solanum species, has previously been shown to have anti-cancer and anti-herpes virus properties [6]. Although considered a weed, *S. elaeagnifolium* is reported to have a broad spectrum of therapeutic properties due to the existence of pharmacologically potential phytochemicals. In order to screen the pharmacological potential of this plant as an antibacterial, anti-inflammatory and antidiabetic agent, the present research aimed to explore its potential using *in-vitro* and *in-silico* strategies.

2. Materials And Methods

2.1. Sample collection and authentication

Before conducting experimental studies, the plant sample was collected in June 2018 in Pithalaipatty Village, Dindigul District to identify the scientific name of the plant. After being prepared as a herbarium, it was then submitted to the taxonomist of the Herbarium Center for Molecular Systematic at St Joseph’s College, Tiruchirappalli for authentication.Later, the specimen was recognized by the taxonomist as *Solanum elaeagnifolium* (Herbarium No.: SB001) (Fig. 1).

2.2. Extract preparation

Mature and un-infected leaves were collected. The collected leaves were later cleaned by running tap water to remove the unwanted particles from the surface of the leaves. In order to prepare the leaves as a fine powder for further research, the leaves were dried in shade environment at 36–40°C for 20 days, which were then loaded into a Soxhlet column for extraction. The solvents such as ethyl acetate, ethanol and acetone were used for the extraction to assess their potential based on polarity.

The following formula was used to compute the extract yield:

$$\mathrm{Yieldofextract}\,(\backslash\%) = \frac{Amountofdriedsample\,(g)}{Amountofextract\,(g)} \times 100$$

2.3. Chemicals and Reagents

In this research, the freshly prepared reagents and chemicals such as Folin Reagent, Ciocleteau's Griess Reagent, Phosphate Buffer, Ferrous EDTA Solution, Nash's Reagent, Sodium Phosphate Buffer, Acetate Buffer, Pnitrophenyl-a-D-glucopyranoside Solution, Dimethyl Sulfoxide (DMSO) and NA- Medium were used to evaluate the antibacterial and antidiabetic enzymatic assays of *S. elaeagnifolium.* To screen the pharmacological potential of the plant extract, exploration was compared to the prescribed drugs such as ascorbic acid for inflammatory activity and acarbose for diabetic enzymatic activity of α-amylase and α-glucosidase enzymes.

2.4. Determination of Total phenolic (TPC) and flavonoid content (TFC)

The TPC in *S. elaeagnifolium* was determined using the Folin-Ciocalteu method according to Amalraj et al. (2021) [7]. 1.0 ml extract was combined with 0.5 ml Folin-Ciocalteu reagent in doses of 20, 40, 60, 80 and 100ml. The mixtures were then kept at room temperature for a few minutes, during which time 2.5 ml of sodium carbonate solution (5%, w/v) were added and mixed. The mixture was left at room temperature for 40 minutes and then the absorbance at 725 nm was measured. To construct a standard curve, gallic acid was used as a standard and the sum of the TPC was expressed as gallic acid equivalents (mg GAE/g). In addition, the amount of TFC was determined by aluminum chloride (AIC13) colorimetry according to Amalraj et al. (2021) [7]. The different concentrations of the extracts were prepared (20, 40, 60, 80 and 100 ml) and the standard quercetin was prepared. 1 ml of extract was mixed with 2 ml of AIC13 and 6 ml of sodium acetate, and then the absorbance at 430 nm was measured. Quercetin was used to plot the calibration curve and its results were expressed as equivalents of quercetin (mg QE/g).

2.4. Antioxidant Activity

2.4.1. Radical scavenging activity using DPPH assay

The DPPH scavenger activity of *S. elaeagnifolium* leaf extracts with methanol was determined using a standard method according to Thangaraj et al [8]. The leaf extracts were formulated with methanol at doses of 50, 100, 150, 200 and 250 g/ml. 100µL of each extracts with various doses was added to 100µL of a 0.1 mM DPPH solution, and then incubated in the dark room at 36°C for 30 minutes. The absorbance was measured at 517 nm. The DPPH concentration in the reaction mixture was determined by a calibration curve (liner regression); using the ascorbic acid as a standard. The scavenging activity of *S. elaeagnifolium* was estimated and expressed as an IC50 value at different doses. The inhibition percentage was calculated by the following equation

$$\backslash\%\text{Inhibition} = \frac{ControlOD - SampleOD}{ControlOD} \times 100$$

2.4.2. Superoxide radical scavenging activity

The superoxide scavenger activity of these plant extracts was calculated by the blue tetrazolium reduction technique [7]. In this test, 100µl of leaf extracts were added to 3 ml of newly prepared reaction mix, 20 g riboflavin, 12 mM EDTA and 0.1 mg NBT in 50 mM sodium phosphate buffer (pH 7,6)) then incubated at 25°C for 5 min and the absorbance was measured at 590 nm. The ascorbic acid in the superoxide scavenger activity was used as a positive control. It was determined by the following equation.

$$\text{Scavengingactivity}\,(\backslash\%) = \frac{ControlOD - SampleOD}{ControlOD} \times 100$$

2.4.3. Ferric reducing antioxidant power (FRAP) assay

It is used in the presence of TPTZ to test the ability of plant extracts to reduce Fe3 + to Fe2 + ions (below pH 3.6) [16]. The freshly prepared FRAP solution was used by mixing 10mM TPTZ in 40mM HCl, 20mM FeCl 3 and 25ml 0.3M acetate buffer (pH 3.6 using 0.3M acetic acid and 0.3M sodium acetate) were mixed in the range of 1:1:10 v/v ratio and the mixture was kept at 37°C until dissolved. First, we take 300µL of leaf extract of different concentration for mixing with FRAP solution. The reaction mixture was incubated at 37°C for 30 min in the dark, where, 593nm was used for absorbance measurement. As a reference, $FeSO_4.7H_2O$ was used in various concentrations such as 10, 20, 30, 40, 50 g/ml and ascorbic acid was used as a standard. The FRAP value was expressed as mol Fe(II) equivalent/mg extract.

2.4.4. Metal chelating activity

The chelating ability on the ferrous ion of *S. elaeagnifolium*was explored according to Amalraj et al [7]. In order to explore the metal chelating activity, the extracts were prepared in various doses such us 50, 100,150, 200, and 250µg/mL.Then the extracts were added to 50 µL of 2 mMFeCl 2 followed by 200 µL of 5 mMferrozine and this solution was made up to 3 mL. Then the reaction mixture was mixed well and kept at room temperature for 10 min. The absorbance of the prepared mixture was measured at 562 nm after the incubation period. Ascorbic acid was used as a positive control. The estimate of the chelating ability in EDTA equivalent (mg EDTA/g extract) is compared to the standard chart.

2.4.5. Total antioxidant activity by phosphomolybdenum assay

The total antioxidant activity of *S.elaeagnifolium* leaf extract was calculated using a phosphomolybdenum assay according to Amalraj et al. (2021) [7]. In this assay, the 100µL of leaf extracts were mixed with 1mL of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate, 4mM ammonium molybdate and the desired volume of 0.6M H_2SO_4. After that, the reaction mixtures were incubated in a water bath at 95°C for 90 min. Then it was cooled to room temperature and the absorbance at 765 nm was measured. Again, ascorbic acid was used as a standard. The results were expressed as equivalent of ascorbic acid per gram of extract (AAE)/g) and higher absorption means high antioxidant ability.

2.5. Antibacterial activity

The antibacterial potential of *S. elaeagnifolium* leaves extract was explored after formulating with ethyl acetate, ethanol and acetone. The prepared extracts were explored against three gram negative bacteria namely *Staphylococcus epidermis* (MTCC435), *Bacillus subtilis* (MTCC441), and *Rhodococcusequi* (MTTC2558) and seven negative bacteria namely *Escherichia coli* (MTCC40), *Shigella flexneri* (MTCC1457), *Salmonella typhi* (MTCC3224), *Pseudomonas aeruginosa* (MTCC1748), *Proteus vulgaris* (MTCC426), *Entero bacteraerogenes* (MTCC8559) and *Vibrio cholera* (MTTC3904).

2.5.1. Minimum inhibitory concentrations (MIC)

Minimum inhibitory concentrations of *S. elaeagnifolium* were determined by a 96-well microdilution strategy [9]. To assess the inhibitory concentration of the tested extracts, 50 ml of plant extracts at different concentrations were used after being serially diluted twice; Pour 50 mL of Mueller Hinton Broth into each well. Then the mixture was incubated for 24 h at 37°C. After 24 hours of incubation, 20 ml of 0.5 mg/ml INT (p-iodonitrotetrazolium or 2-[4-iodophenyl]-3-[4-nitrphenyl]-5-phenyltetrazolium chloride) was added to each well

and resuspended for 30 min. Then the mixture was incubated at 37°C. After incubation, wells treated with plant extracts were compared to the ampicillin. Finally, the wells treated with the S. elaeagnifolium leaf extracts were observed after application of the INT dye.

2.6. *In vitro* anti-diabetic assays

The *in-vitro* enzymatic anti-diabetic assay was used to determine the inhibition of the α-amylase and α-glucosidase potential of *S. elaeagnifolium* leaves according to Lordon et al. [10].This was achieved using starch and p-nitrophenyl-a-D-glucopyranoside (PNPG) as substrates, using the acarbose as the reference drug.

2.6.1. Inhibitory assay of α-amylase

In this assay, 100ml of *S. elaeagnifolium* leaves extracts were incubated at 25°C for 10 minutes at different concentrations with 1% starch solution in a 20 mM phosphate buffer (pH 6.9 with 6 mMNaCl). Then 100 ml of the enzyme amylase (0.5 mg/ml) was added and the mixture was incubated at 25°C for 10 minutes. Later, 200 ml of dinitrosalicylic acid reagent was added to stop the hydrolytic reaction, and the reaction mixture was incubated at 100°C for 5 minutes. The samples were then cooled to room temperature and then the 50 ml reaction mixture was transferred to a microplate (96 wells). Then it was diluted by adding 200 ml of distilled water. The absorbance was estimated at 540 nm. Ultimately, the range of α-amylase inhibition of the *S. elaeagnifolium* extract was determined as follows,

$$\%\text{Activity} = \frac{Absorbanceofextract}{Absorbanceofcontrol} \times 100$$

2.6.2. Inhibitory assay of α-glucosidase

In order to the explore the inhibitory potential of α-glucosidase using *S. elaeagnifolium* leaves extracts, a 100 mM sodium phosphate buffer (pH 6.9) was mixed with 50 ml of *S. elaeagnifolium* extracts. Later it was combined with 50 ml of 5 mM PNPG solution (in a phosphate buffer) to induce a hydrolytic reaction. The mixture was incubated at 37°C for 5 min and then a phosphate buffer (100 ml) containing 0.1 U/ml α-glucosidase was added to each well. After 30 min, the absorbance at 405 nm was measured and the percentage of α-glucosidase inhibition of these extracts was calculated as follows:

$$\%\text{Activity} = \frac{Absorbanceofextract}{Absorbanceofcontrol} \times 100$$

2.7. Gas chromatography mass spectroscopy (GC-MS)

Shimadzu (QP2020) is used to carry out the GCMS analysis of SELEinterfaced to a mass spectrometer. In this spectrometer the SH-Rxi-5Sil-MS is nonpolar, capillary column of 30 m length, 0.25 mm inner diameter and the film thickness is 0.25 mM coated with 100% polydimethyl-siloxane. Initial temperature of oven was maintained at 50°C and increased to 280°C at a rate of 6°C/min with 2 min final hold time. The temperature of the injector was 250°C. At a linear velocity of 39.7 cm/sec and a pressure of 68.1 kPa, the carrier gas helium was used at a flow rate of 1.2 mL/min. With a split ratio of 1:10, one mL of leaf extract dissolved in hexane was injected into GC. The mass spectrum was obtained at 70 eV in EI mode and the mass spectrum was estimated in the range

of 50 to 500 amu.At 200°C, the ion source temperature was preserved. Compound interpretation was achieved by comparing the mass spectrums of compounds found in leaf extracts of SE with normal spectra in the MS library of NIST 2005 and in literature [11]. By measuring the average peak area for the total area in the chromatogram, the relative percentage of compounds was determined.

2.8. In silico anti-diabetic validation

Prior to the *in-silico* research, the phytochemicals of the leaves of *S. elaeagnifolium* were identified using GC-MS. To understand the anti-diabetic potential of this plant, they were docked to the diabetic proteins using the Schrödinger suite. For the *in-silico* validation, the diabetes proteins were retrieved from the protein database. The alphanumeric identity of these targets is 1B2Y, 1H5U, and 1QL6. The spectrally detected phyto-ingredients were retrieved from the Chemspider as a mol file. After retrieving the sources from the established databases, they were prepared using Maestro V13.2, in which the ligand was prepared using the ligprep tool and the protein was prepared using the protein preparation wizard tool. After the preparation of the ligands and proteins, the prepared molecules were docked to the Glide XP module. The entire in silico process was carried out according to Prabhu et al. [12] and Kalaimathi et al. [13].

3. Results And Discussion

3.1. Yield of crude extracts:

The overall yield of crude extracts from *S. elaeagnifolium* leaves using ethyl acetate, ethanol and acetone solvents was expressed in percent, namely 4.77%, 6.80% and 4.94%, respectively (Table. 1). Based on its polarity, percentage of total yield of crude and presence of phytochemicals in the plant extracts further investigations such as enzymatic and antioxidant properties of *S. elaeagnifolium* extracts were also investigated.

3.2. Total phenolic and flavonoid content

Total phenolic content was calculated using the standard curve linear regression equation (y = 0.0222 × -0.0414, R2 = 0.9646) and total phenolic content was evaluated as milligrams per gram equivalent of gallic acid (Table. 1). In this quantitative phytochemical exploration, the ethanol extract yielded the utmost amount of total TPC (158.77 ± 1.46mg/g GAE), followed by ethyl acetate (79.04 ± 0.98mg/g GAE) and acetone (41.78 ± 0.14 mg/g GAE). Similar to the TPC, the total flavonoid content was calculated using the standard curve linear regression equation (y = 0.0319 × -0.05254, R2 = 0.9792) and total flavonoid content was determined as milligrams per gram equivalent of quercetin (Table. 1). In TFC exploration, ethyl acetate extract had the highest amount of TFC (134.31 ± 0.04mg/g QE), followed by ethanol (120.03 ± 0.73mg/g QE) and acetone extracts (116.39 ± 0.48mg/g QE).

3.4. Antioxidant activity of Solanum eleagnifolium

3.4.1. DPPH radical scavenging assay

Antioxidant activity of extracts in terms of hydrogen donating or free radical scavenging capacity was calculated using the DPPH method. DPPH scavenging is expressed as an IC50 value, which is defined as the

sample concentration required to elicit 50% inhibition and was calculated by interpolation from a linear regression analysis. A higher radical scavenger activity is correlated with a lower IC50 value [14]. The present research found that the highest antioxidant activity was observed in the ethyl acetate formulation. The IC50 values of the tested extracts are 16.62 ± 0.25g/mL in ethyl acetate, 40.32 ± 0.12g/mL in ethanol and 103.81 ± 0.47g/mL in acetone, respectively, as shown in Table 2.In 2014, a study by Houda et al. [15] found that the highest inhibition (IC50) was found in immature fruits of *S. elaeagnifolium*, particularly in the methanol extracts.

3.4.2. Superoxide radical scavenging test

The plant extract showed the utmost antioxidant activity in the ethanol extract. The IC50 for scavenging free radicals was 33.96 ± 4.5g/mL in ethanol, 19.61 ± 1.61g/mL in ethyl acetate and 16.13 ± 11.05g/mL in acetone extracts, respectively (Table – 2). In general, the superoxide anion is detrimental to cellular components. Previously, Robak and Glyglewski [16] reported that the flavonoids are powerful antioxidants because they scavenge superoxide anions. Consistent with this statement, the present research found that the plant extract formulated with ethanol extract has a more effective scavenger of superoxide radicals than the standard used because of its flavonoid content. Similar to the present research, some other species of the genus Solanum such as *Solanum aethiopicum* (61.5%), followed by *Solanum macrocarpon (*45.8%) and *Solanum torvum* (35.2%) were reported to have the highest hydrogen peroxide scavenging activity and strong antioxidant potential [17–19].

3.4.3. Ferric Reducing Antioxidant Power Assay (FRAP)

The sample reduces the iron (III) cyanide complex to the iron (II) form (Fe^{2+}). The FRAP value in the extract of the ethyl acetate was found to be 995.67 ± 50.82 (Table 2). It was observed to be higher than the acetone and ethanol extracts. The ethyl acetate extract shows the higher FRAP value compared to the other two formulations. The result of the present research came close to the result of Johan et al [20]. Particularly, the superoxide was remarkably inhibited by plant extract at maximum dose level. More specifically, the ethyl acetate of the plant extract has maximum activity at the highest concentration and has been shown to be an effective superoxide radical scavenger.

3.4.4. Phosphomolybdenum assay

The total antioxidant activity of the plant extract is shown in Table 2. The notable results were observed in acetone extracts at 168.67 ± 1.92 E/g and followed by ethanol (109.78 ± 10 E/g) and ethyl acetate (100.70 ± 9.97 E/g). Maximum total antioxidant activity was provided by SE ethanol extract (19.4 mg/mL), followed by *L. camara* ethanol extract (18 mg/mL), *L. camara* petroleum ether extract (3.4 mg/mL), and *S .elaeagnifolium* (1.5 mg/ml) at 1000 mg/ml [21]. Whereas the methanolic extract of adiantum and caudatum leaves showed much higher activity than the hexane and aqueous extracts, while the hexane extract was the least active. This order of activity from polar methanol to non-polar hexane extract is quite normal as the polar solvents have a much stronger ability to dissolve and thus extract polar phytochemicals [22].

3.4.5. Metal chelating activity

The antioxidant capacity of *S. elaeagnifolium* was determined by a metal chelate assay. The notable inhibition was observed in the plant extract formulating with acetone (663.50 ± 2.1E/g) followed by ethyl acetate (539.33

± 6.67 E/g) and ethanol (445 .44 ± 3.47 E/g) (Table. 2). This finding reinforces the notion that the metal chelating activity of the ethyl acetate extract from *S. anguivi* showed higher metal chelating activity. The scavenging potential and metal chelating ability of the antioxidants depend on their unique phenolic structure and the number of hydroxyl groups [23]. The metal ion chelating capacity (% inhibition of Fe2+-ferrozine complex formation) at a concentration of 1000µg/mL of ethanol, hexane and aqueous extracts was found to be approximately 64, 45 and 41%, respectively.

3.5. Minimum inhibitory Concentration of S. elaeagnifolium

In order to know the minimum inhibitory dose of *S. elaeagnifolium* leaves, the explored extracts were tested on the bacterial strains. Table 3 clearly showed the inhibitory potential of the extracts in a dose dependent manner. Among the strains tested, a remarkable inhibitory potential was found in the extract formulated with ethanol at the doses of 29.687 and 59.375 g/ml against *Pseudomonas aerogenosa* and *Vibrio cholera*. Conversely, the lowest inhibition potential of the formulated extracts was also found in numerous strains at a dose of 950 g/ml, which is entered in the table. 3. The inhibitory potential of the extract is known by the wells turning red-pink, confirming bacterial growth, and no color, confirming no microbial growth. Consistent with this statemnt, the MIC of extracts has been precisely displayed in Fig. 2. Previous studies have reported that the differences in inhibitory concentration at a dose-dependent level may be due to the solubility of the chemical content of the extracts in solvents, particularly on a polarity basis. Generally, the phytochemicals are soluble in the solvents and therapeutic potentials also unfold either alone or synergistically with other phytochemicals from formulated extracts [24].

3.6. In vitro Anti-diabetic Activity of SELE

The results of in-vitro anti-diabetic activity revealed α-amylase activity possess the best inhibition than α-glycosidase activity (Fig. 3).

3.6.1. α-amylase inhibition activity

The plant extracts were formulated with the solvents ethanol, ethyl acetate and acetone, which exploited their anti-diabetic potential by inhibiting α-amylase activity. The range of inhibition of each formulation has been shown in Table.1.As shown in Table 1, the extract formulation of acetone and ethyl acetate solvents were found as notable α-amylase inhibitors. The inhibitory concentration of extracts was defined as the sample concentration required eliciting 50% inhibition, at which point the higher inhibition is recorded as the IC50 value. At the end of this analysis, the healthier IC50 values were found in the ethanol extract. Similar to the present research, Selvi and Yoganath [25] previously investigated the anti-diabetic potential of stems and leaves of this genus species, namely *S. nigrum*. Their research precisely shows that the percentage of inhibition of α-amylase is greater as the concentration of extract treatment increases. They reported that it could be happened due to the enzyme α-amylase converts starch into glucose.In 2014, Suhashini et al. [26] reported that the plant extracts showed enzyme inhibitory potential when the α-amylase and glucose were taken together with the plant extract as a solution. In particular, the plant extracts such as *Solanum torvum* and *S. trilobatum* were treated after formulation with water; where, antidiabetic activity has been revealed by these extracts.

3.6.2. α-glycosidase activity

Leaves of this plant were used for the α-glycosidase inhibitory activity after formulated with solvents such as acetone, ethyl acetate and ethanol. Finally, the results showed that the formulation of ethanol extract has the maximum inhibition activity with 66.87% at 50 g/ml and the result is expressed as an IC50 value. The present research revealed that the significant IC50 of α-glycosidase in ethanol extract was found to be 27.90 g/mL (Table. 1). According to the research results, the present research has found that the leaves of this plant have potential anti-diabetic activity, showing that the plant extracts have potential activity against α-glycosidase enzyme. The α-glycosidase was significantly inhibited in a dose-dependent manner. Previous studies have reported that the Solanum species have shown significant pharmacological potential in in vitro and in vivo assessment when tested for anti-diabetic, antimicrobial and hemolytic activity [2, 27, 28]. Consistent with this statement, one species of Solanum is currently identified as having pharmacological properties for antibacterial, antioxidant and anti-diabetic.

3.7. GC-MS analysis of Solanum elaeagnifolium leaves Cav.

In this research, nearly 23 phytochemicals were detected in the ethanol-formulated plant extract by GC-MS (Table. 4), which was examined to determine if the molecules were actively acting as anti-diabetic agents. Table 4 clearly shows the percentage of peak area and the molecular formula of the detected phytochemicals of this plant extract in ethanol solvents (Table. 4). GC-MS detected the phytochemical solanesol, previously reported to have inhibitory potential against *E. coli* and *S. aureus* [29]. In 2013, a study screened the phytochemicals in *Solanum elaeagnifolium*, which are quite close to the phytochemical detected in the present research, but they have employed the solvents in contrast to present research [30].

3.8. In silico molecular docking studies

To identify the active antidiabetic molecules in the leaves of *Solanum elaeagnifolium*, the phytochemicals detected by GC-MS were used for molecular docking with three diabetic proteins. Furthermore, to rank the anti-diabetic potential of ligands, the phytochemicals were docked collectively with three conventional drugs against diabetic proteins. The marketed drugs selected in this study are prescribed to diabetics around the world.

3.8.1. 1B2Y with Phytoconstituents of S. elaeagnifolium

Prior to the docking, the active ligand binding pocket was identified which was used for fixing grid as drug target site. It has numerous amino acids and also possesses leading site scores as a possible ligand binding site (Fig. 4). This docking complex assessment found the phytochemical cyclolaudenol to have leading docking scores of -7.943 with energy scores of -41.847 (Table. 5). Similarly, campesterol and Stigmasta-5,22-dien-3-ol have significant values comparable to cyclolaudenol (Fig. 5a & b). Conversely, the diabetic drugs such as acarbose, metformin and glipizide have the lowest docking scores as well as the lowest contacts. Ultimately, the research shows that the phytochemicals such as betulin and tocopherol possessed remarkable values that were quite similar to acarbose; however, they are also superior to metformin and glipizide in terms of docking scores and binding affinities with human pancreatic alpha-amylase.

3.8.2. 1H5U with Phytoconstituents of S. elaeagnifolium

Tocopherol was found to be a suitable active ingredient for diabetes in the docked complex of glycogen phosphorylase B with phytochemicals and diabetes drugs, which was supported by its docking results (Fig. 6).

It has leading docking scores of -7.414 with the energy values of -46.661 than other ligands including diabetic medicines (Table. 6). Because no phytochemicals other than tocopherol have been shown to interact with this protein, they cannot be taken for further research line related to diabetes. It has almost 232 binding nodes with the amino acids of 1H5U (Fig. 7). On the other hand, acarbose 178 binding nodes were shown as binding affinities; whereas the metformin was found to have 62 binding nodes (Fig. 8a and 8b). Acarbose has a docking score of 7.332 among antidiabetic drugs, which is quite close to tocopherol. However, glipizide was not involved in 1H5U, meaning it has no activity, whereas metformin only displayed negligible docking values (Table. 6). The binding affinity nodes were shown in green color.

3.8.2. 1QL6 with Phytoconstituents of S. elaeagnifolium

After examining the docked complex of phytochemicals and diabetes protein, tocopherol was identified as a suitable active ingredient for problem related to phosphorylase kinase in diabetics. It has leading docking scores of -7.253 with the energy values of -46.395 (Table 7). Figure 9 shows the ligand pose in the binding pocket of phosphorylase kinase. As a possible drug candidate for phosphorylase kinase, Tocopherol has almost 178 binding nodes (Fig. 10a). Likewise, the campesterol was found to have the second best docking score of -6.964 with the energy scores of -43.466 (Fig. 10b). This was found to have 152 binding nodes. On the other hand, commercial drugs such as acarbose, glipizide, and metformin were found to have moderate docking scores, which are clearly shown in Table 7. Acarbose has 175 binding affinity nodes while glipizide has 135 binding affinity nodes with the amino acid residues of this 1QL6 (Fig. 11a & b).

4. Conclusion

In the current scenario, finding drugs without side effects and a biological molecule that does not change as a medium for microorganisms growth remains a very difficult task. Since the advent of drug-resistant strains, microbes, particularly bacteria, are causing serious health problems in humans. The bacterial infections can be found in the diabetics by an unpleasant appearance with clinical syndromes such as erythema, pain, tenderness and pus. Since bacterial infection is quite natural in diabetics, the present research aimed to investigate the antioxidant, antibacterial and anti-diabetic (Inhibition of α-amylase and **α-glycosidase enzyme**) potential of *S. elaeagnifolium* leaves. In order to know the active anti-diabetic phytochemicals of this plant, the phytochemicals were assayed on diabetic proteins using an *in silico* approach. The plant extract formulated with ethanol solvents was found to have remarkable antibacterial potential against *E. coli, Proteus vulgaris, Staphylococcus epidermis, Bacillus subtilis* and *Rhodococcusequi*. The minimum inhibitory concentration of this formulation on these bacteria was found at a dose of 118.75 g/ml. On the other hand, the remarkable α-amylase and α-glucosidase enzyme inhibitory potential was observed in the extract formulated with plant extract. This computational research showed that phytochemicals such as cyclolaudenol, campesterol and tocopherol exhibit remarkable docking values and strong binding affinity nodes with diabetic proteins in addition to the side chain and backbone contacts of amino acids. Based on the *in-silico* research, we conclude that the phytochemicals may have exerted their antidiabetic potential in in vitro research either alone or synergistically with other phytochemicals from this plant. The significant discovery of this study is that the plant has triple pharmacological potential including antioxidant, antibacterial and antidiabetic properties; therefore, the present research strongly suggests that this plant could be used for drug development. Last but

not least, we hope this research could be a mentor to researchers, especially those interested in working on this plant.

Abbreviations

SE, *Solanumelaeagnifolium*; TPC, Total phenolic content; TFC, Total flavonoid content; DPPH, 2,2 –Diphenyl-1-picrylhydrazyl radical scavenging activity; SO, Superoxide radical scavenging activity; FRAP, Ferric reducing antioxidant power assay; MC, Metal chelating assay; PHM, Total antioxidant activity by phosphor-molybdenum assay, MIC, Minimal inhibitory concentration

Declarations

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Funding

Not Applicable

Availability of data and material

During the current study the datasets used and analyzed materials will be getting from first and corresponding authors on reasonable request.

Authors' contributions

TFX did the conception of the work, data analysis, interpretation and critical revision of the manuscript. **RS** and **SB** done the sample collection and extract preparation. **RK, AKFR** and **MA** helped in designing the experimental work and also contributed in the preparation of the manuscript. **SV** assisted in the molecular docking research. **SP** took part in data interpretation of molecular docking, modeling, language revision and polishing of the manuscript. Finally, all authors read and approved the manuscript. **SA** assisted in the paper collection and manuscript preparation.

Consent for publication

Not Applicable

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Tables

Table.1: Yield, Total phenolic and flavonoid content, α-amylase and α-glucosidase activities of *S. elaeagnifolium* leaf extracts

Solvents	Yield of extract %	TPC mg/g GAE[$]	TFC mg/g QE[#]	α-amylase IC_{50} (µg/ml)*	α-glucosidase IC_{50} (µg/ml)*
Ethyl acetate	4.77	79.04±0.98	134..31±0.04	$41.56±1.63^{ab}$	$59.76±13.66^{ab}$
Ethanol	6.80	158.77±1.46	120.03±0.73	$17.78±2.38^{a}$	$27.90±5.02^{a}$
Acetone	4.94	41.78±0.14	116.39±0.48	$17.96±6.05^{a}$	$36.44±3.30^{b}$
Acarbose	NA	NA	NA	15.28±0.43	14.51±0.23

[$] *milligram per gram equivalent of gallic acid*

[#] *milligram per gram equivalent of quercetin*

** The Inhibitory concentration of the sample required to inhibit the enzymes activity by 50%. The values of result mentioned as means (n=3) ± standard deviation. Same letters as superscript in same column (for different*

*extracts/samples); **NA:** Not Applicable*

Table. 2: Total antioxidant activity of *S. elaeagnifolium* leaf extracts

Solvents	DPPH	SO	FRAP	PHM	MC
Ethyl acetate	16.62±0.25^{a}	19.61±1.61^{b}	995.67± 50.82^{c}	100.70 ± 9.97^{a}	539.33±6..67^{e}
Ethanol	40.32±0.12^{ab}	33.96±4.5^{a}	278.52± 42.87^{c}	109.78 ± 10.60^{a}	445.44±3.47^{c}
Acetone	103.81±0.47^{ab}	16.13±11.05^{b}	847.10± 28.58^{c}	168.67±1.92^{a}	663.50± 10.93^{e}
Ascorbic acid	13.23±0.35	11.34±0.75	1127.10 ± 11.64	147.56 ± 0.0	357.39±2.10

Values in IC_{50} (µg/mL); DPPH - Radical scavenging activity using 2,2-diphenyl-1-picrylhydrazylmethod; SO - Superoxide radical scavenging activity (µg/ml); PHM - Phosphomolybdenum assay (mg/g); FRAP - Ferric reducing-antioxidant power assay (mM Fe (II) E/mg); MC - Metal chelating activity (EDTA E mg/g). Values are expressed as mean (n=3) ± standard deviation; same letters as superscript in same column (for different extracts/samples) are not significantly different according to Tukey's test ($p<0.0001$).

Table-3: Antibacterial activity of *S. elaeagnifolium* leaf extracts by minimum inhibitory concentration

Formulated Extracts	Minimum inhibitory concentration (µg/ml)									
	EC	***PV***	***SE***	***BS***	***RE***	***VE***	***ST***	***SF***	***PA***	***EA***
Ethyl acetate	237.5	118.75	475	237.5	>950	475	950	475	>950	>950
Ethanol	118.75	118.75	118.75	118.75	118.75	59.375	950	118.75	29.6875	59.375
Acetone	237.5	118.75	475	237.5	>950	475	475	237.5	>950	>950
Ampicillin	7.5	7.5	7.5	7.5	7.5	7.5	1.875	3.75	3.75	7.5

EC - *E.coli*; PV - *Proteus vulgaris*; SE - *Staphylococus epidermis*; BS - *Bacillus subtilis*; RE - *Rhodococcusequi*; VC – *Vibrio cholera*; ST - *Salmonella typhi*; SF – *Shigella flexneri*; PA - *Pseudomonas aerogenes*; EA –*Enterobactera eroegenes*.

Table. 4: GC-MS analysis of *S. elaeagnifolium* ethanol leaf extract

Peak	Retention time	% Peak area	Molecular Mass	Name of the Compound
1	5.589	0.2	101	N,N-diethyl-Formamide,
2	6.942	1.14	115	N,N-diethyl-Acetamide,
3	7.691	0.89	108	Benzenemethanol
4	17.522	0.16	518	Tetradecamethyl-Cycloheptasiloxane,
5	20.769	0.38	592	Hexadecamethyl-Cyclooctasiloxane,
6	23.574	1.54	666	Octadecamethyl-Cyclononasiloxane,
7	26.067	0.24	740	Icosamethylcyclodecasiloxane
8	26.517	0.24	278	1,2-benzenedicarboxylicacid,dibutylester
9	28.347	0.37	592	Tetracosamethyl-Cyclododecasiloxane,
10	28.928	18.09	414	Gamma-sitosterol
11	31.166	30.86	630	Solanesol
12	31.75	1.74	290	Geranyllinaloolisomerb
13	32.804	19.36	546	Lycopersene
14	34.641	0.96	330	Hexadecanoic acid, 2,3-dihydroxypropyl ester
15	34.877	0.48	390	1,2-Benzenedicarboxylicacid, diisooctylester
16	34.983	0.75	272	M-Camphene
17	35.634	1.13	368	1-Pentacosanol
18	36	0.84	430	Alpha-Tocopherol
19	36.13	2.04	426	Alpha-amyrin
20	36.228	2.19	442	Betulin
21	38.418	3.44	400	Campesterol
22	38.61	1.99	440	Cyclolaudenol
23	39.108	6.03	412	Stigmasta-5, 22-dien-3-ol

Table. 5: Molecular docking assessment of Phytochemical and conventional drugs as possible anti-diabetic molecule against human pancreatic alpha-amylase (PDB ID: 1B2Y)

S. No.	Name of the phytochemicals	Docking Scores	Energy	Being prescribed as anti-diabetic drugs
1.	Cyclolaudenol	-7.943	-41.847	No
1.	Campesterol	-7.875	-40.392	No
1.	Stigmasta-5,22-dien-3-ol	-7.868	-39.773	No
1.	Acarbose	-6.866	-48.566	**Yes**
1.	Sitosterol	-6.706	-31.527	No
1.	Betulin	6.243	-42.575	No
1.	Tocopherol	-6.017	-37.452	No
1.	amyrin	-3.803	-40.495	No
1.	Glipizide	-3.538	-21.394	**Yes**
1.	Metformin	-4.706	-31.527	**Yes**

Table.6: Molecular docking assessment of phytochemical and conventional drugs as possible anti-diabetic molecule glycogen phosphorylase B (PDB ID: 1H5U)

S. No.	Name of the Phytochemicals	Docking Scores	Energy	Being prescribed as anti-diabetic drugs
1.	Tocopherol	-7.414	-46.661	No
1.	Glipizide	-7.338	-67.515	**Yes**
1.	Metformin	-3.116	-26.082	**Yes**

Table.7: Molecular docking assessment of phytochemical and conventional drugs as possible anti-diabetic molecule Phosphorylase kinase (PDB ID: 1QL6)

S. No.	Name of the phytochemicals	Docking Scores	Energy	Being prescribed as anti-diabetic drugs
1.	Tocopherol	-7.253	-46.395	No
1.	Campesterol	-6.964	-43.466	No
1.	Acarbose	-5.166	-62.334	**Yes**
1.	Stigmasta-5,22-dien-3-ol	-5.143	-33.466	No
1.	Glipizide	-4.535	-52.631	**Yes**
1.	Cyclolaudenol	-3.652	-26.676	No
1.	Betulin	-3.073	-13.119	No
1.	Metformin	-3.007	-22.402	**Yes**

Figures

Figure 1

Authenticated specimen of *Solanum elaeagnifolium* Cav.

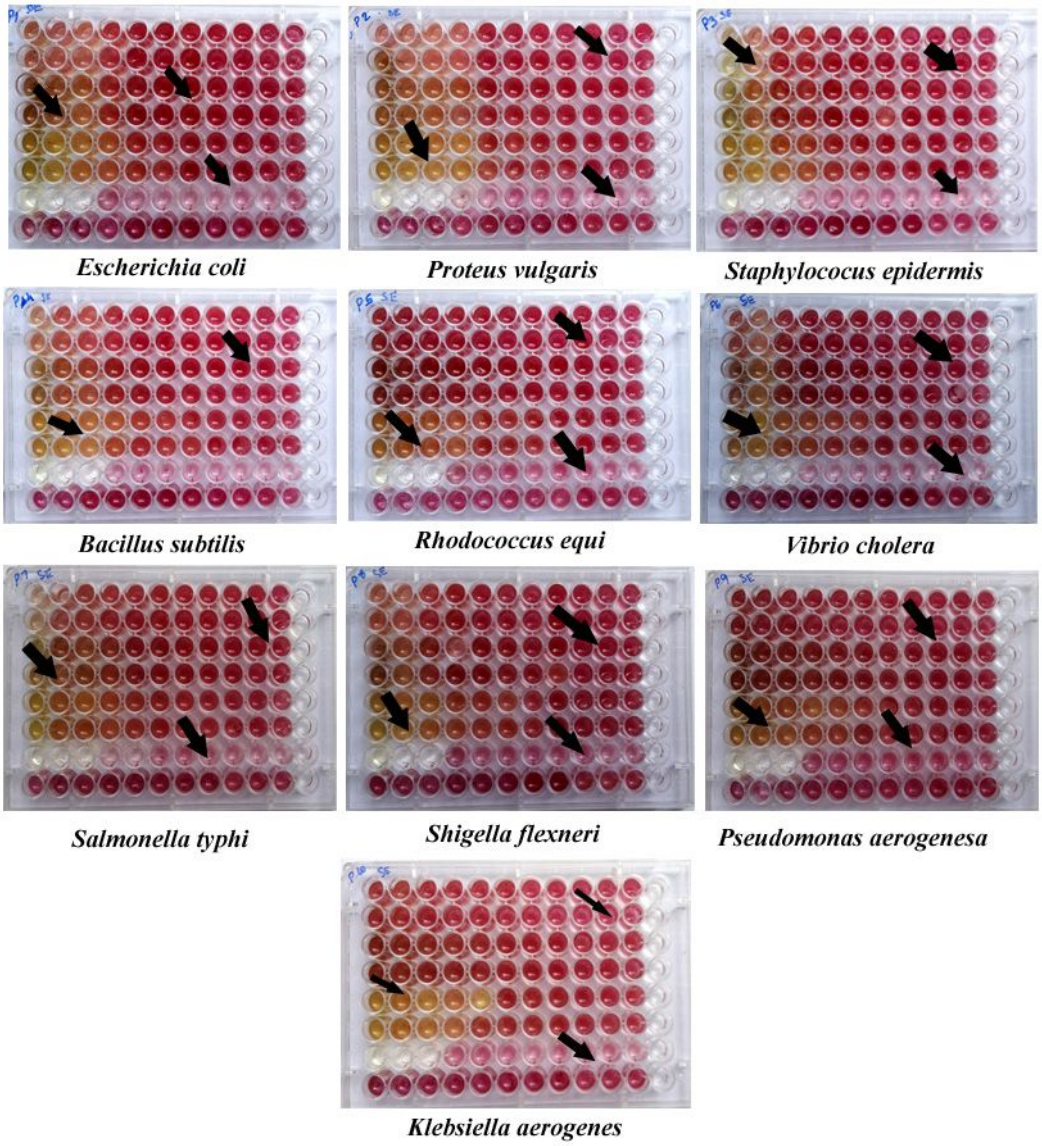

Figure 2

The minimum inhibitory concentration range of *S. elaeagnifolium* extracts in different solvents

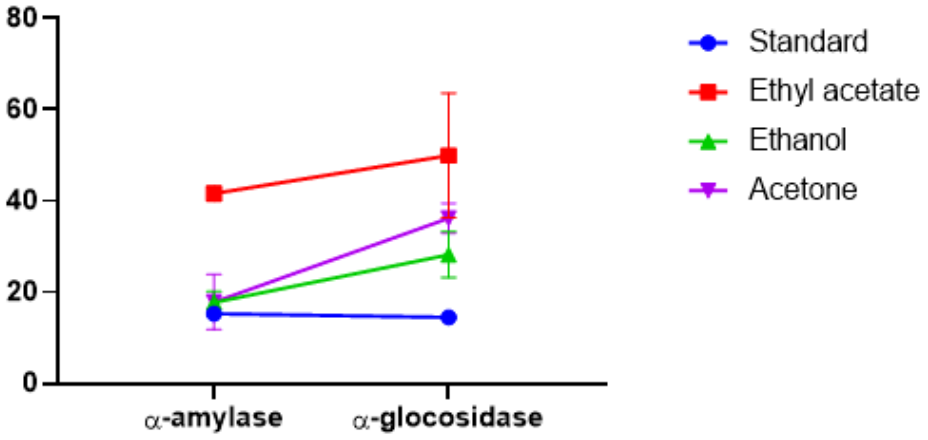

Figure 3

α-amylase and α-glucosidase inhibitory activity of *Solanum elaeagnifolium*leaf extracts at different concentrations. Values are expressed as means ± SD (n = 3).

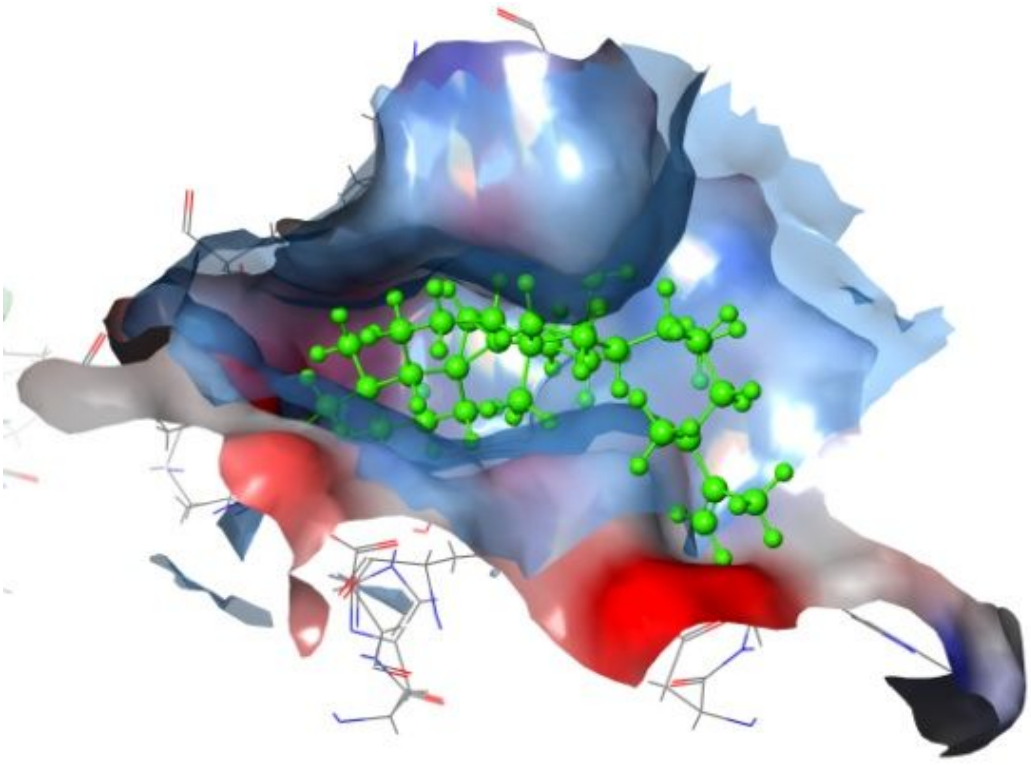

Figure 4

Active ligand binding Pocket of 1B2Y

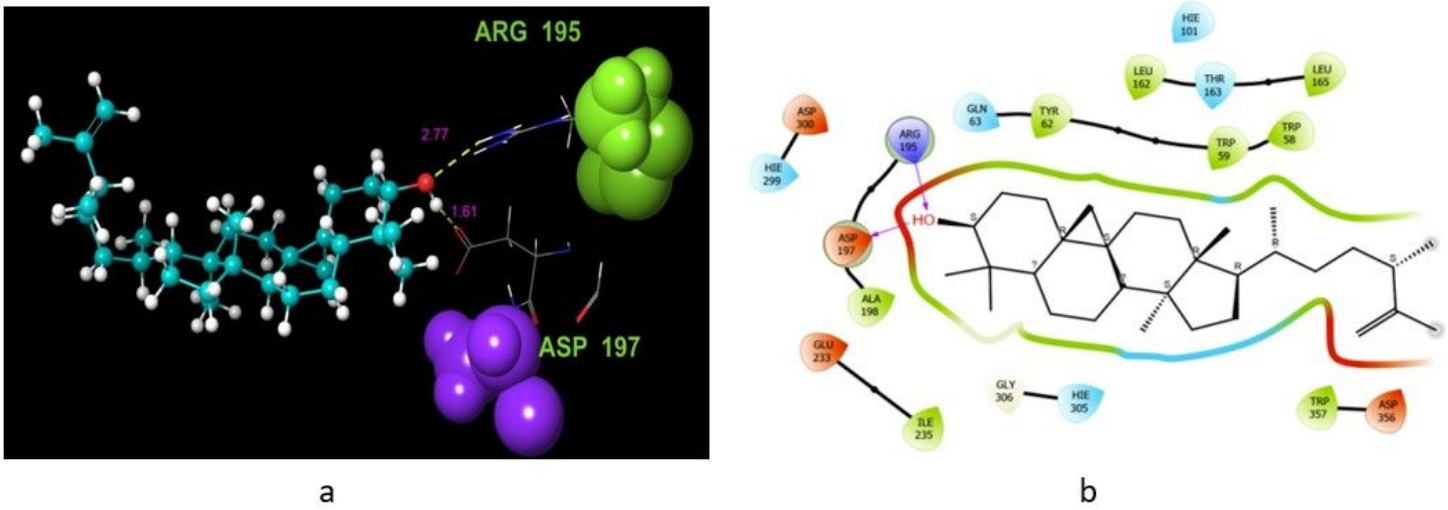

Figure 5

Fig.4a) **Cyclolaudenol:** Residues and hydrogen bond contacts with their distance values with 1B2Y protein and b). The 2D template shows the sort of interactions that exist between the hydroxy groups of Cyclolaudenol with 1B2Y

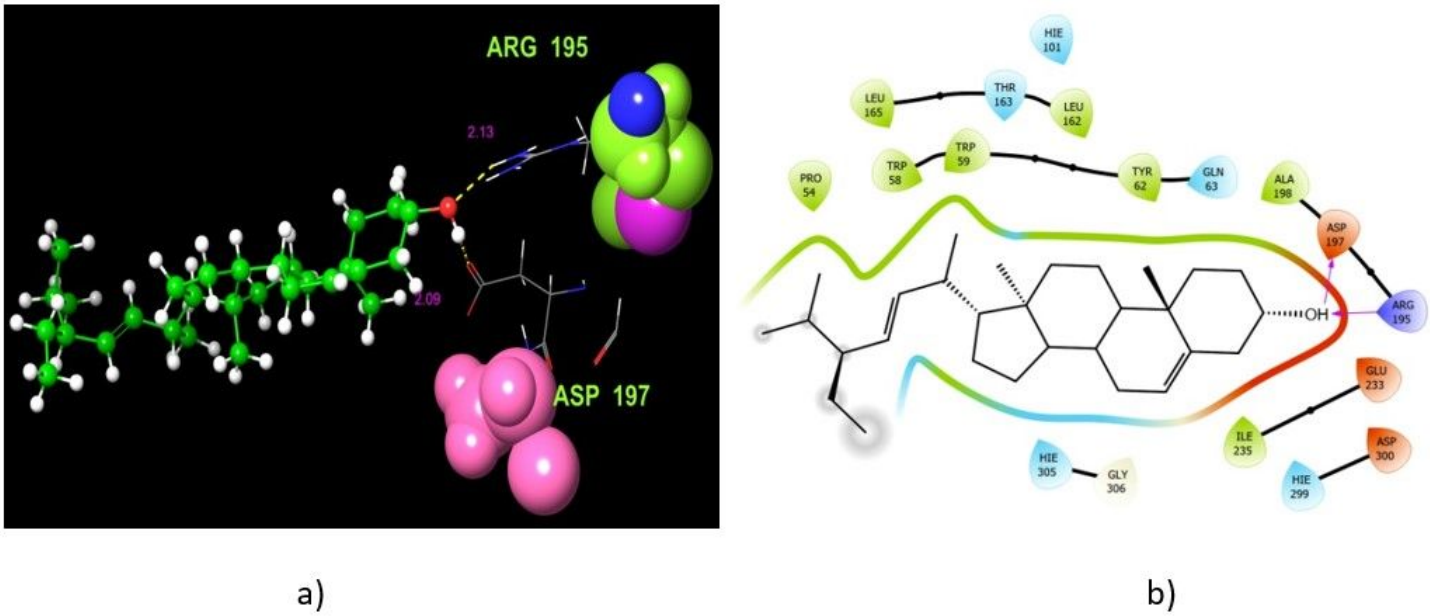

Figure 6

Fig.5. a) **Stigmasta-5,22-dien-3-ol:** Residues and hydrogen bond contacts with their distance values with 1B2Y protein and b). The 2D template shows the sort of interactions that exist between the hydroxy groups of Stigmasta-5,22-dien-3-ol with 1B2Y

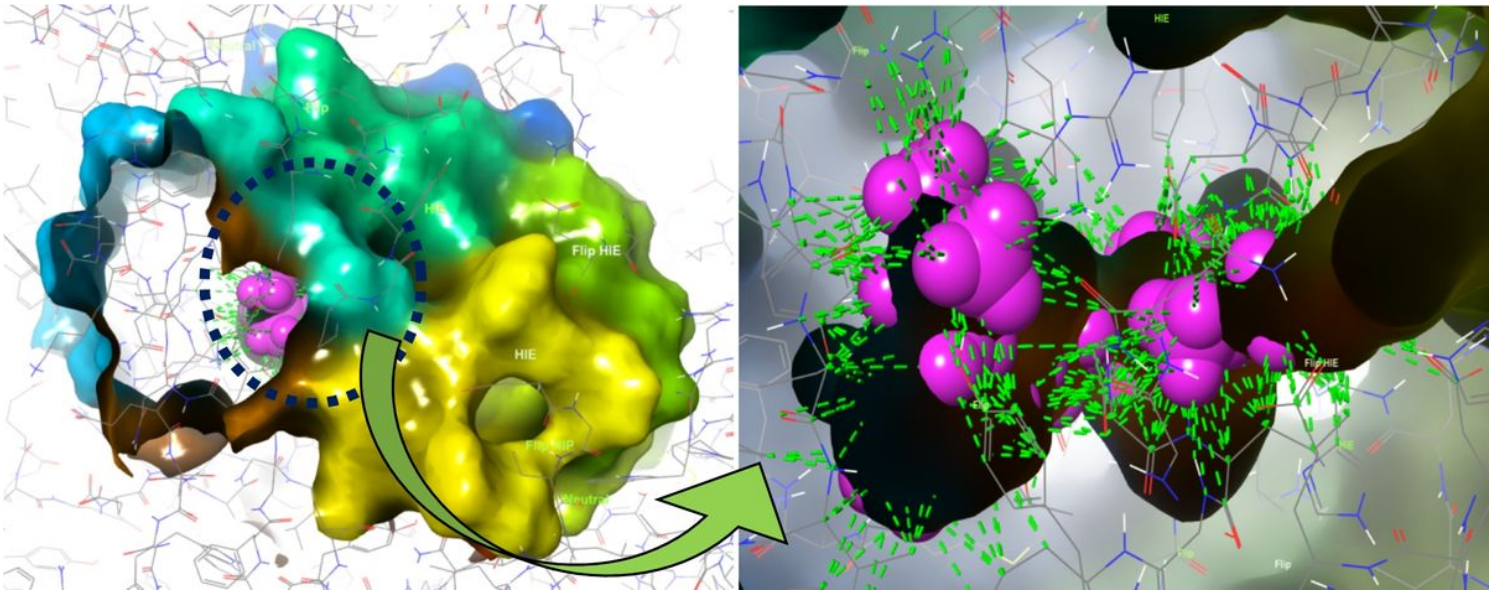

Figure 7

Fig.6 Active ligand binding Pocket of 1H5U

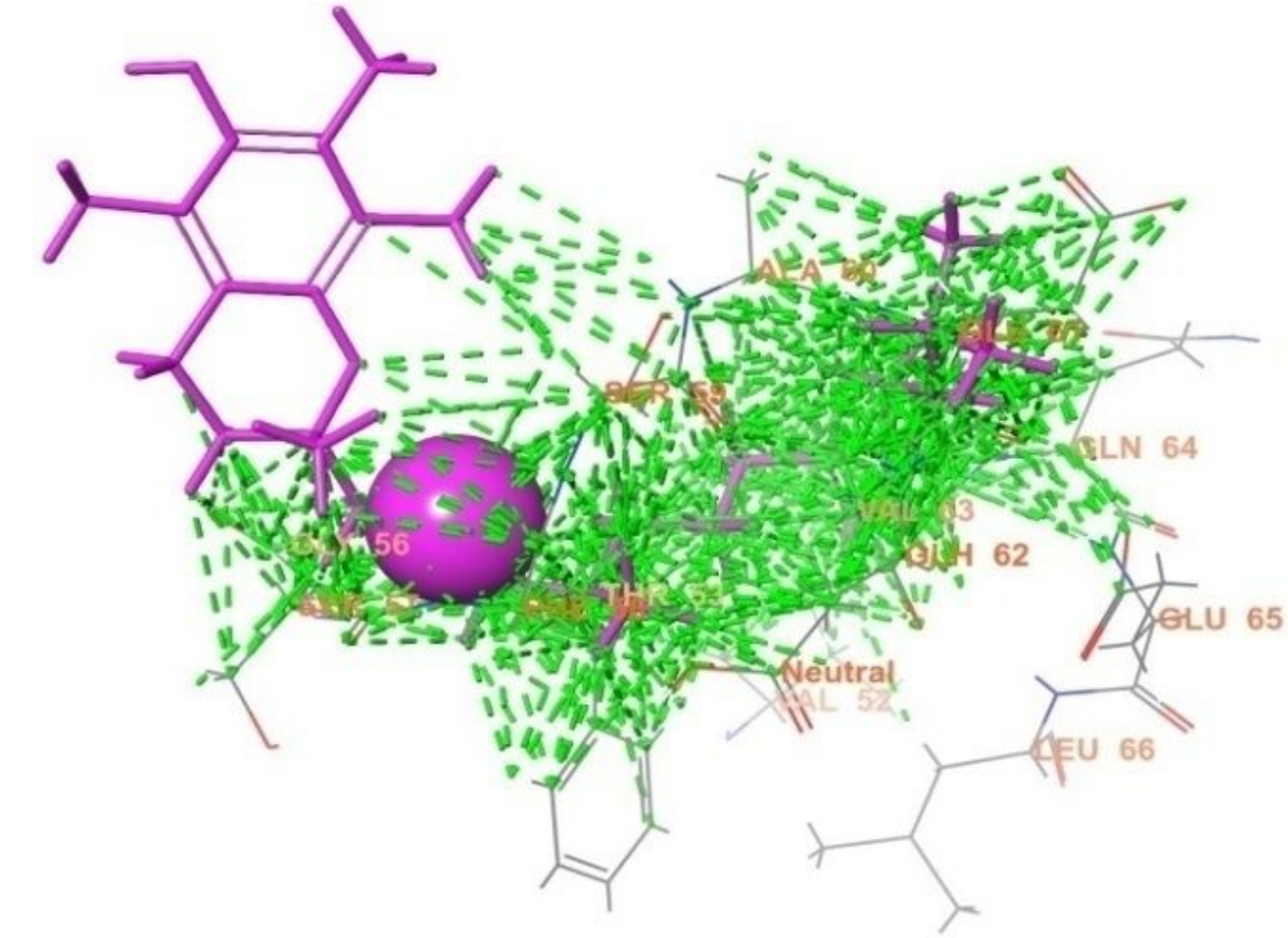

Figure 8

Fig.7. **Tochopherol:** Contacts lines of Binding affinities with the residues of 1H5U

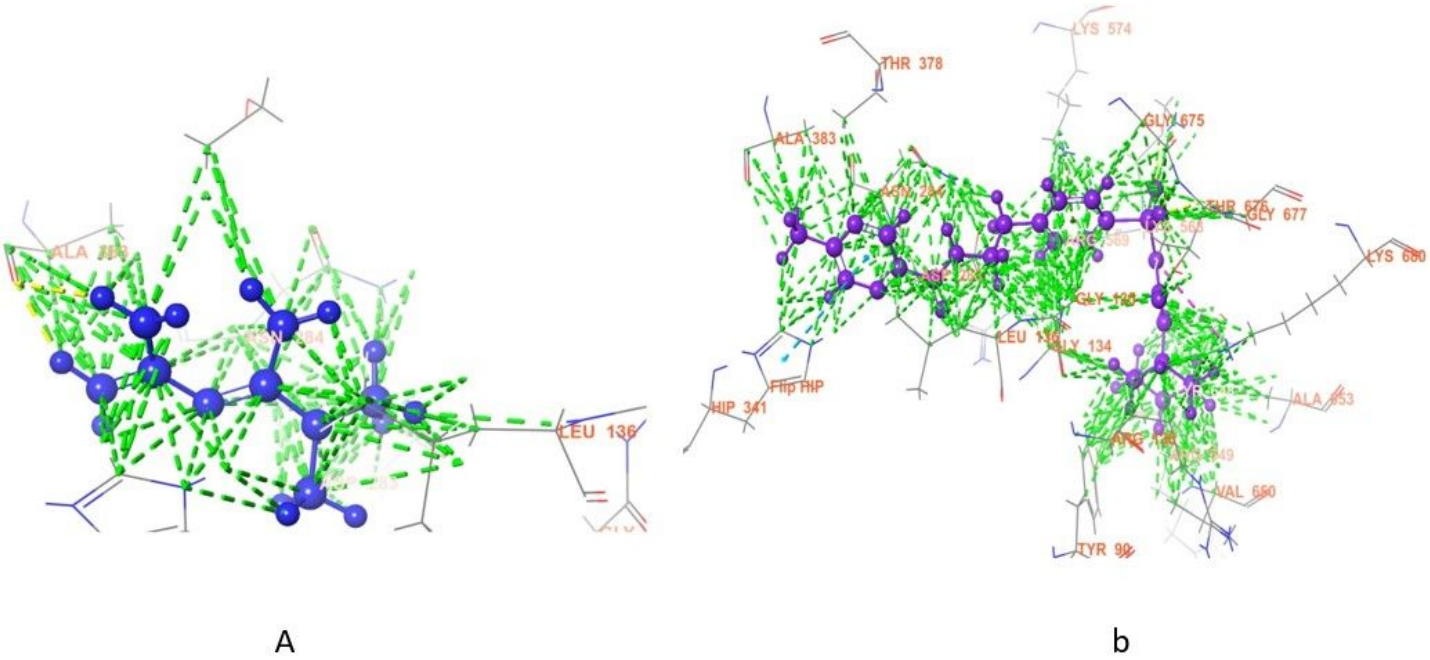

Figure 9

Fig.8**a). Metformin** and **b).Glipicide:** Contacts lines of Binding affinities with the residues of 1H5U

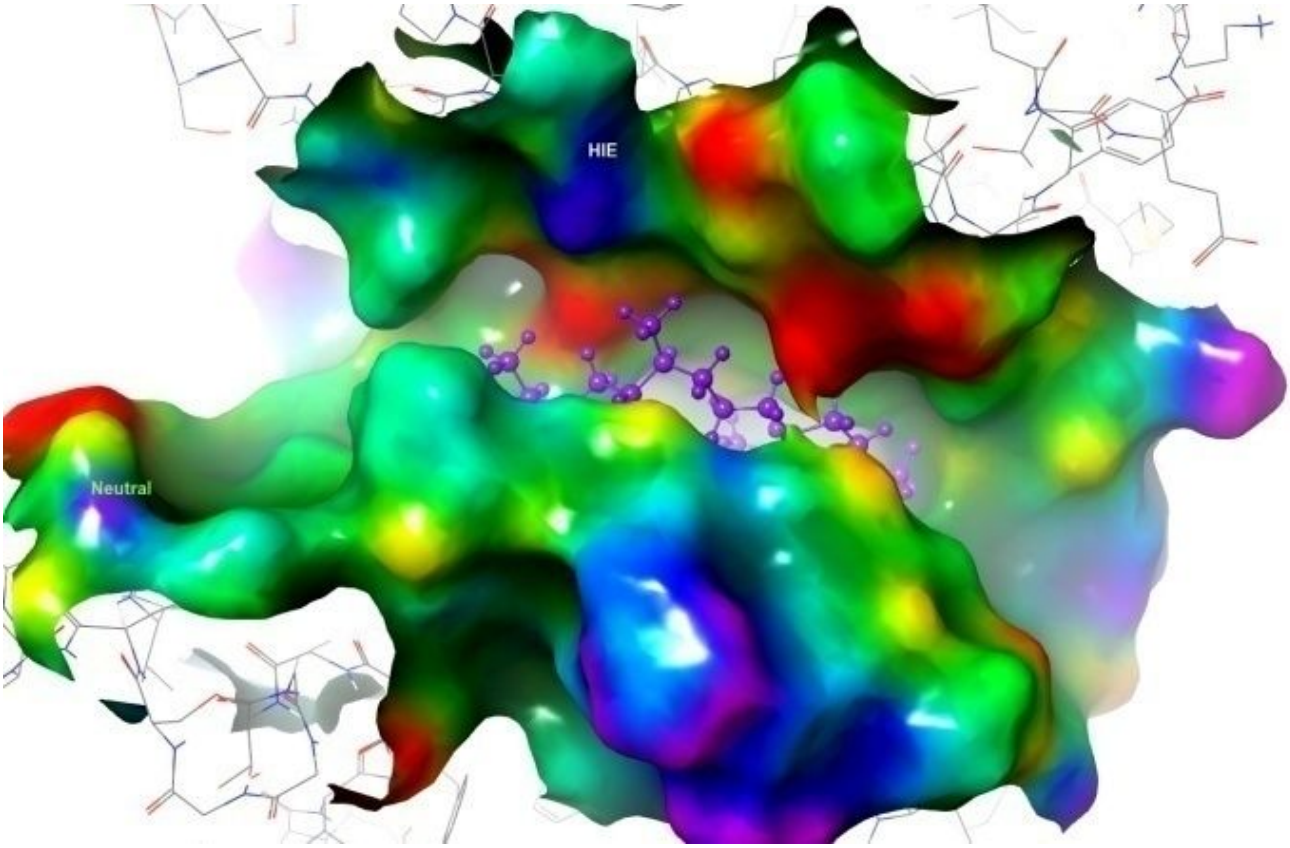

Figure 10

Fig.9. Active ligand binding Pocket of 1QL6

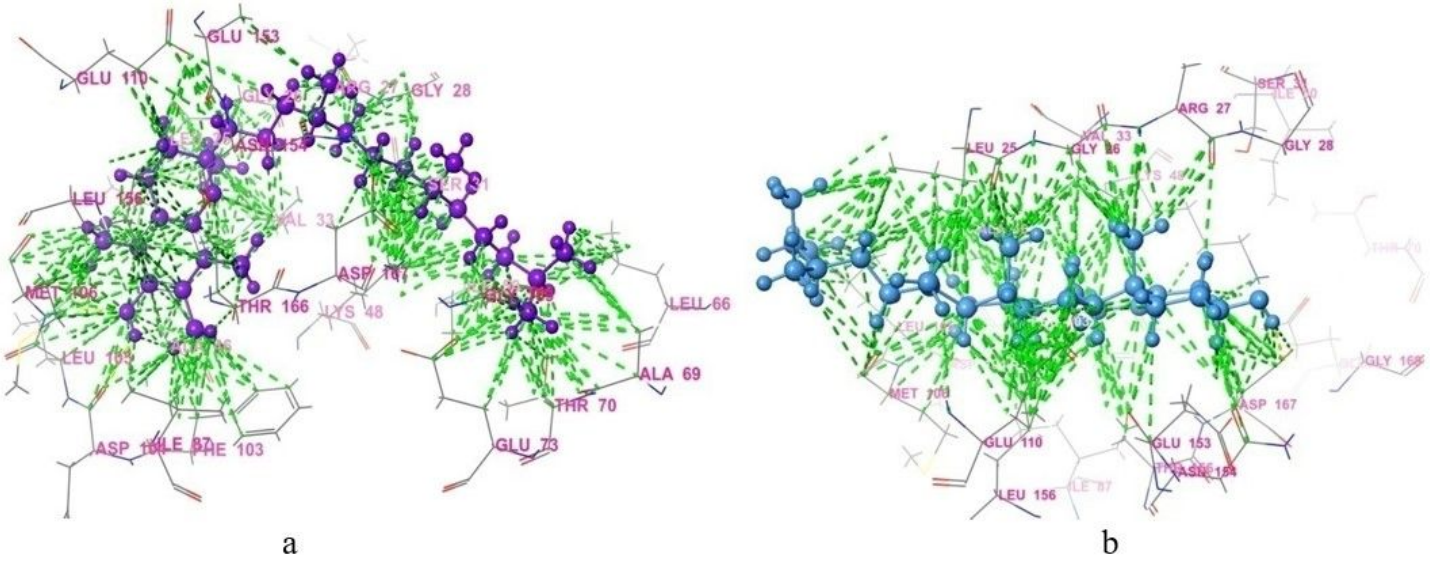

Figure 11

Fig. 10**a).Tocopherol**and **b).Campesterol:** Contacts lines of Binding affinities with the residues of 1QL6

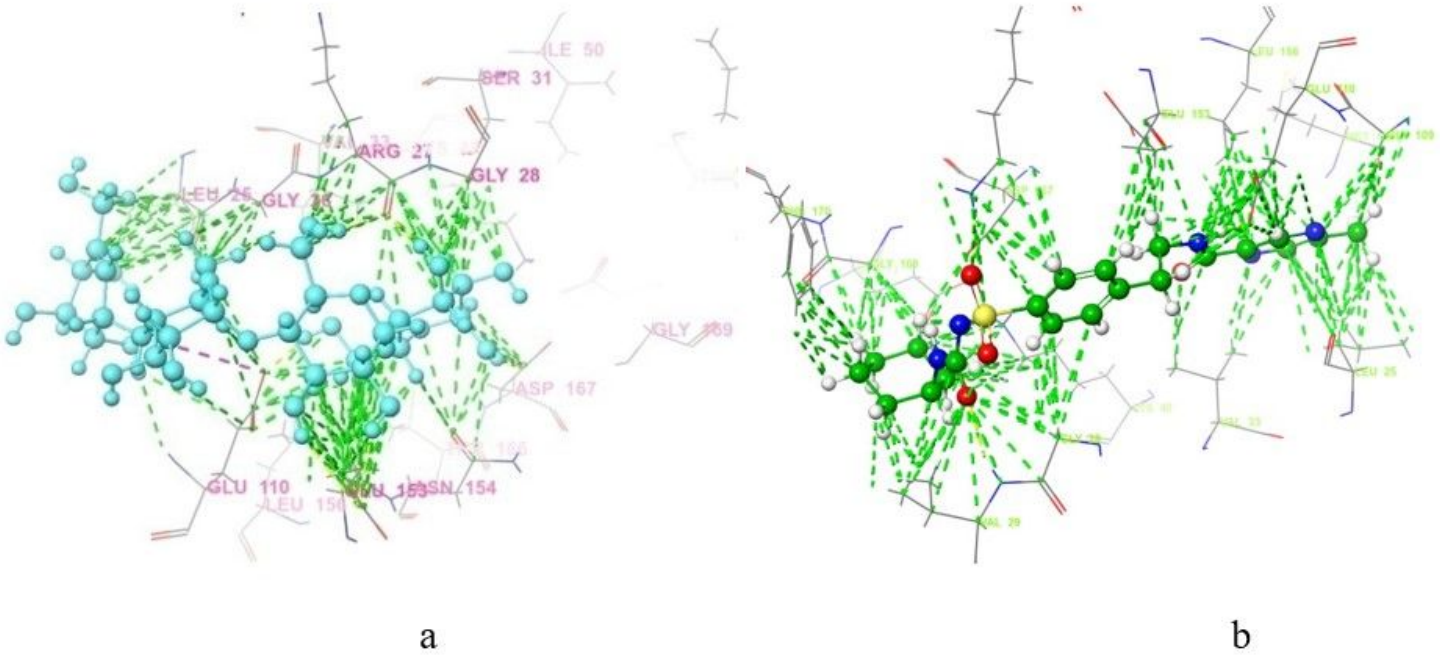

Figure 12

Fig. 11**a).Acarbose**and **b).Glipizide:** Contacts lines of Binding affinities with the residues of 1QL6