Jordan Journal of Pharmaceutical Sciences, Volume 15, No. 2, 2022

Antidiabetic activity, polyphenols-based characterization and molecular interaction of extract of un-ripe pods of *Vinca rosea* cv. Pink

Abida Qamar [1 *]

[1] Punjab University College of Pharmacy, University of the Punjab Lahore, Pakistan

ABSTRACT

The study aimed to investigate extracts of un-ripe pods of Vinca rosea cv. Pink for antidiabetic activity and polyphenol-based characterization of extracts using HPLC. Moreover, the molecular interaction of the identified markers with the antidiabetic targets was explored using molecular-docking software. Different extracts of un-ripe pods were prepared and investigated using antidiabetic models such as glucose uptake by yeast cells, alpha amylase inhibition and haemoglobin-glycosylation inhibition assays. The most active extract was characterized by HPLC using chlorogenic, caffeic and ferrulic acids as an analytical markers. The identified markers were taken as ligands for molecular docking with pancreatic α-amylase, glycogen phosphorelase and hexokinase-I using 1-Click Docking Mcule Software, and finding hydrogen-bonding affinities by UCSF Chimera 1.12. Methanol extracts showed higher antidiabetic activity of 37.77, 71.16 and 53.52% inhibition of α-amylase assay, increase in glucose-uptake by yeast cells, and inhibition of Hb-glycosylation assay, respectively. The extract was found to contain 0.25 mg/g of chlorogenic and 0.11 mg/g caffeic acids. These markers were found to be good ligands of diabetes related targets. The results indicate that methanol extract has antidiabetic activity, which may be assigned to chlorogenic and caffeic acids. These compounds may be used as pharmacological markers to standardize the extracts.

Keywords: Vinca rosea cv. Pink, Antidiabetic activity, Un-ripe pods, polyphenols, Reversed-phase HPLC, Molecular interaction.

INTRODUCTION

Plants, being the source of diverse bioactive compounds, may play a key role in controlling diabetes, which is affecting more than 170 million individuals globally. [1-2] For such purpose, medicinal plants used as folklore antidiabetic medicine need to be explored to find alternative remedies for this alarming disorder. As a folk-remedy, people swallow fresh leaves of *Vinca rosea (*synonym: *Catharanthus roseus* (L.) G. Don) by chewing to control diabetes. The literature also provided evidence that extracts of leaves of the plant had antidiabetic activity. [2-3-4] But, to the best of our literature review, extracts of un-ripe pods of *Vinca rosea* cv. Pink have not been investigated for antidiabetic activity.

Vinca rosea is commonly called Madagascar periwinkle, Rose periwinkle and cape periwinkle belongs to the family *Appocynaceae*. It is an evergreen herbaceous plant, usually 1m in height having long green glossy leaves arranged in opposite pairs on slender stem. Flowers are usually white to rose pink with a darker red centre. The plant is reported to contain a number of phenolic compounds [5-6] some of which (chlorogenic, caffeicand ferulic acids) have been investigated for their antidiabetic action. Wang et al.[7]had investigated the effects of chlorogenic acid on the hepatic glucose 6-phosphatase,blood glucose, skeletal muscles GLUT4 expression and lipid level in streptozotocin-induced diabetic

[*]***Corresponding author: Abida Qamar***
abidabilal23@gmail.com
Received on 10/3/2021 and Accepted for Publication on 12/11/2021.

rats. This report indicated that chlorogenic acid may ameliorate the changes of glucose metabolism, insulin sensitivity, glucose 6-phosphatase expression, lipid metabolism and skeletal muscle GLUT4 expression in streptozotocin-induced diabetic rats. The antidiabetic effect of caffeic acid and ferulic acid was also investigated in steptozotocin-induced diabetic mice. [8-9]Caffeic and ferulic acids are naturally occurring hydroxycinnamic acids while chlorogenic acid is family of esters of hydroxycinnamic acids (caffeic, coumaric,sinapic and ferulic acids) with quinic acid.[10]These polyphenols may be used as pharmacologically active analytical markers to characterize or standardize extracts/extract-based products. Therefore, these markers were selected to characterize the active extracts of un-ripe pods of the plant for the first time. The identified markers were used to investigate the molecular interaction of these compounds as ligands with targets proteins.

To provide possible mechanism of action, bioinformatics tools are becoming important to understand the interaction of ligands with different disease-targets.[11])

Among these tools molecular interactions are gaining much attention and these can be studied using the molecular docking approach. Molecular docking can be used to model the interaction between ligand and target protein at atomic level. Docking is not possible with the extracts as these are mixtures of several known and un-known chemical compounds. Such studies are possible by selecting marker compounds which are present in the extract. Several drug-targets could be used to control diabetes but based on the model used to investigate antidiabetic activity, α-amylase, glycogen phosphorelase and hexokinase- I were selected as drug-targets to determine binding affinity of the markers. The results of the present study may be beneficial for finding evidence-based alternative remedy for controlling diabetes.

RESULTS AND DISCUSSION

The results of antidiabetic activity of methanol extract (ME), chloroform extract (CE) and petroleum ether extract (PE) using different models are given in Figure 1.

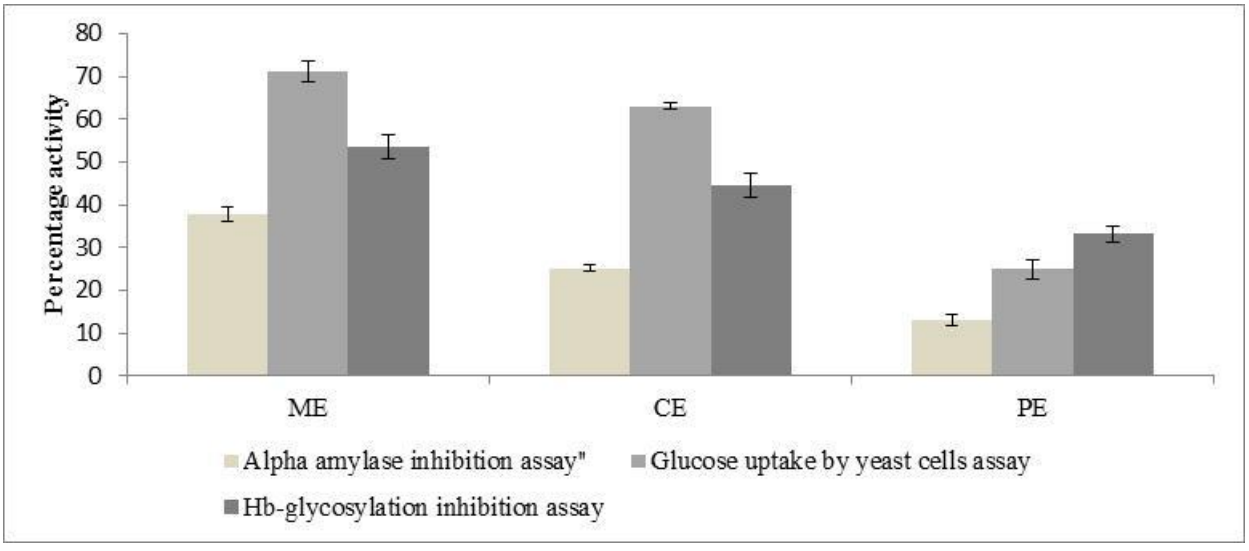

Figure 1. Antidiabetic activity of methanol (ME), chloroform (CE) and petroleum ether (PE) extracts of un-ripened pods of *Vinca rosea* by α-amylase inhibition, glucose uptake by yeast cells and Hb glycosylation inhibition methods

ME, CE and PE of un-ripe pods had shown 37.77, 25.61 and 3.06% activity, respectively, to inhibit α-amylase. The α-amylase is one of the main secretory enzymes of pancreas (about 5–6%) and salivary glands which play a role in the digestion of starch and glycogen. As it is reported by Lo-Piparoet al. [12] that inhibition potential is correlated with the number of hydroxyl groups present in the B ring of polyphenol ligands which form hydrogen bonding between hydroxyl group and catalytic residues of the binding site of enzyme. Therefore, the inhibition of this enzyme is considered a strategy to manage disorders related to carbohydrate uptake such as diabetes, obesity and periodontal diseases. [13]

In the second applied antidiabetic model, the effect of extracts (ME, CE, PE) was observed on the uptake of glucose inside the yeast cells. The ME of un-ripe pods had shown 71.16% uptake of glucose by yeast cells while CE and PE had shown 63.05% and 24.87% uptake of glucose, respectively. The transport of glucose inside the yeast cells is a complicated process which is facilitated by carriers that transport the glucose down the concentration gradient. It means intracellular removal of glucose molecule is necessary to attain the effective transport. [14] If most part of the sugar present inside is converted into metabolites, the internal level of glucose will be down and higher uptake of glucose will be favored. So, there might be possibilities that in the presence of extracts, the uptake of glucose inside the yeast cells took place either by facilitated diffusion and or elevated glucose metabolism.

The extracts of un-ripe pods also have shown significant inhibition of Hb glycosylation. The ME had resulted in to 53.52%, CE with 44.65% and PE with 33.16% inhibition of Hb glycosylation. In glycosylation, glucose reacts non-enzymatically with amino group of Hb through series of reactions resulting into the formation of advance glycated end products (AGEs). The unnecessary accumulation of these AGEs is believed to be responsible for chronic complications of diabetes. [15] The formation of free radicals, reactive carbonyl and dicarbonyl groups take place through series of reaction of glycosylation. Therefore, capturing the free radicals at the early stage of glycosylation leads to decrease the production of reactive carbonyl and dicarbonyl groups which in turn can inhibit the glycated end products. [16] According to Adisa et al. [17] plant products may likely inhibit the formation of AGEs and free radicals in diabetes which implies the reduction of oxidative stress in diabetes. The extracts of un-ripe pods might contain antioxidant phytochemicals which might contribute the anti-glycation effect. The overall results had depicted that ME is an active extract of un-ripe pods of *Vinca rosea* than CE and PE ($p < 0.05$).

Polyphenols-based characterization of ME by HPLC

The analytical markers such as chlorogenic acid, caffeic acid and ferrulic acid were used to characterize ME by HPLC method. The chromatogram of the mixed standards (Figure 2) indicated that peaks of all the compounds were well-resolved.

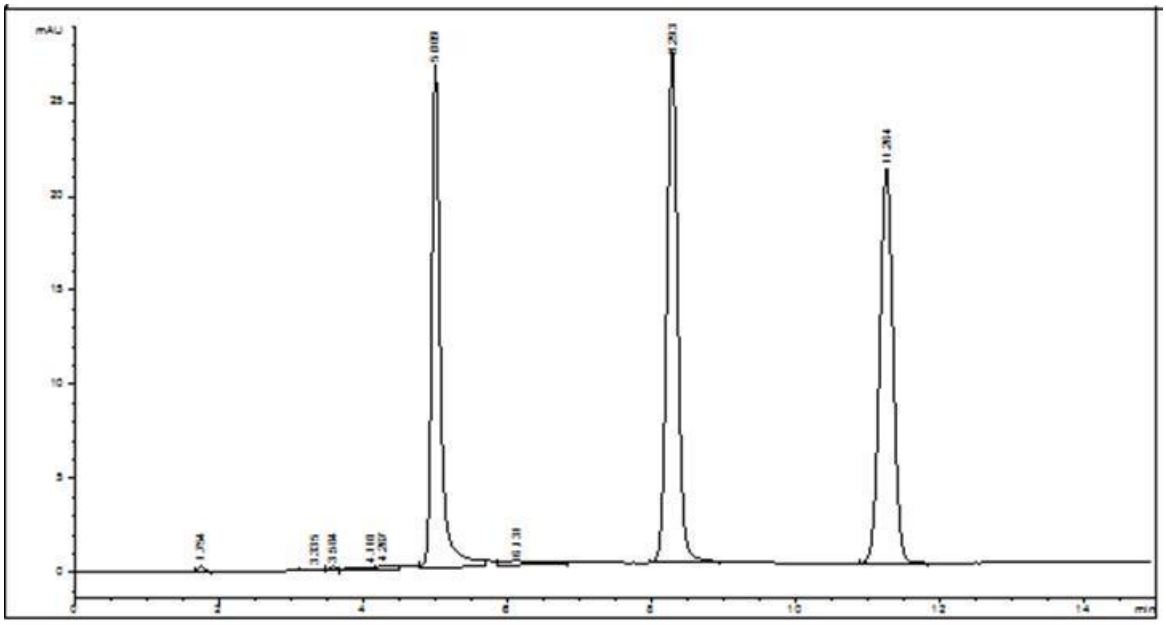

Figure 2. The chromatogram of standards chlorogenic acid (retention time, 5.00), caffeic acid (retention time, 8.29) and ferrulic acid (retention time, 11.264) respectively

Moreover, the peaks of all the standards were Gaussian, hence used to determine system suitability parameters which are given in Table 1.

Table 1. System suitability parameters of the method for the determination of chlorogenic acid, caffeic acid and ferulic acid

Parameters	Chlorogenic acid	Caffeic acid	Ferulic acid	Limit
Theorical plates (N)	17530.47	52335.23	70135.17	> 2000
Capacity factors (k')	5.02	8.28	11.26	≥ 2.0
Tailing factors (As)	1.005	1.00	1.002	≤ 2
Resolution (Rs) of chlorogenic acid and caffeic acid	21.751			≥ 1.5
Resolution (Rs) of caffeic acid and ferulic acid	18.839			≥ 1.5

Theoretical plate (N) is an index that indicates column efficiency
Capacity factor (k') is used to help assess if a peak is going to give reproducible and linear results over time
Tailing factors (As) shows the degree of peak symmetry
Resolution (Rs) is the measure of separation of two peaks of different retention time t

These results show that system is suitable for the analysis of a mix solution of chlorogenic acid, caffeic acid and ferulic acid. The method used for characterization of extract was found to be linear over the whole range investigated with the linear regression equations for chlorogenic acid, caffeic acid and ferulic acid, y = 20.97x +13.96, y =45.56x+16.91, y = 60.2x + 31.32 with correlation coefficient R^2 =0.997, R^2 = 0.996, R^2 = 0.991, respectively. Limit of detection and limit

of quantification for the markers were found to be 0.078572 and 0.238096 µg/mL for chlorogenic acid, 0.023803 and 0.072132 µg/mL for caffeic acid, 0.053463 and 0.162008 µg/mL for ferulic acid. Recovery, intraday and inter-day and accuracy for chlorogenic acid, caffeic acid and ferulic acid were found in a range of 90-110% with relative standard deviation less than 5%.

The chromatogram of ME (Figure 3) indicated two peaks of chlorogenic acid (5.02 min) and caffeic acid (8.29min). The peak areas of these peaks were used to determine contents of chlorogenic acid and caffeic acid using the linear regression equation of chlorogenic acid (y = 20.97x +13.96, R^2 =0.997) and caffeic acid (y =45.56x+16.91, R^2 = 0.996).

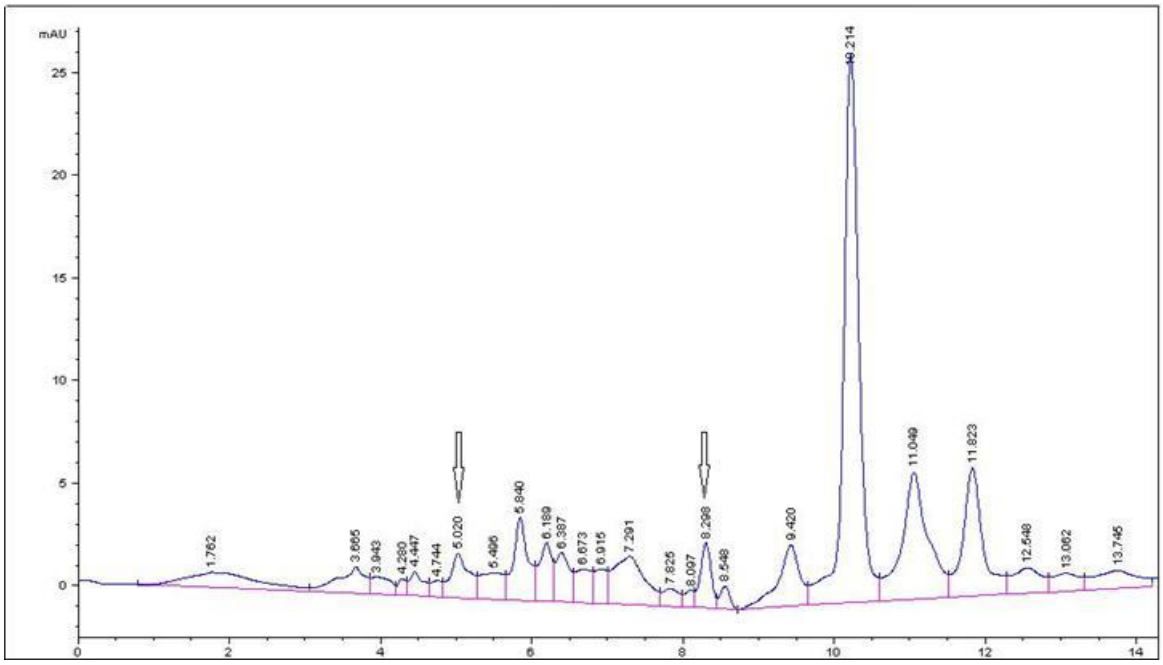

Figure 3. The chromatogram of methanol extract of un-ripened pods of *Vincarosea*showing peaks with retention time 5.02(chlorogenic acid) and 8.29 (caffeic acid) respectively

The amounts of chlorogenic acid and caffeic acid were found to be 0.245 and 0.11 mg/g, respectfully. However, ferulic acid was not detected in ME. Pereira et al. [18] and Nishibe et al. [19] have reported the presence of chlorogenic acid and caffeic acid in the leaves of *Vincarosea*.

Molecular docking studies

The docking studies on the active site of alpha amylase, glycolgenphosphorelase and hexokinase- I were performed by the 1-Click Docking Mcule and UCSF Chimera 1.12 Programs which had shown ligand-target binding modes in terms of lowest docking energy. The target proteins were docked with polyphenols such as chlorogenic acid and caffeic acid, excellent results were seen by lower values of binding energy. The best possible binding mode showing hydrogen bonding of ligands (chlorogenic acid and caffeic acid) with target enzymes are shown in the Figure 4A and 4B; 5A and 5B; 6A and 6B, respectively. Alpha amylase proteins residues ILE235. A, ASP 197.B, ASP 300.A, HIS 305.A have formed four hydrogen bonds with chlorogenicacid. Caffeic acid also has formed one hydrogen bonding with protein residues ARG 195.A. The binding energies and bond distances for both ligands are shown in Table 2.

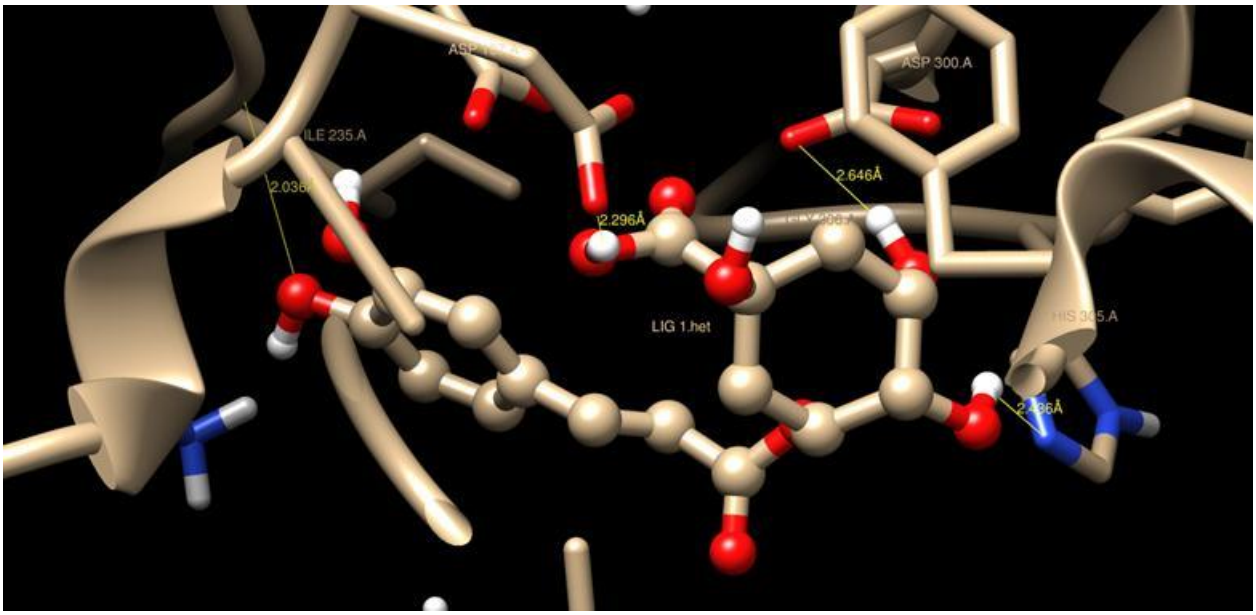

Figure 4A. The predicted structure details of hydrogen bonding of alpha-amylase residues with ligand chlorogenic acid

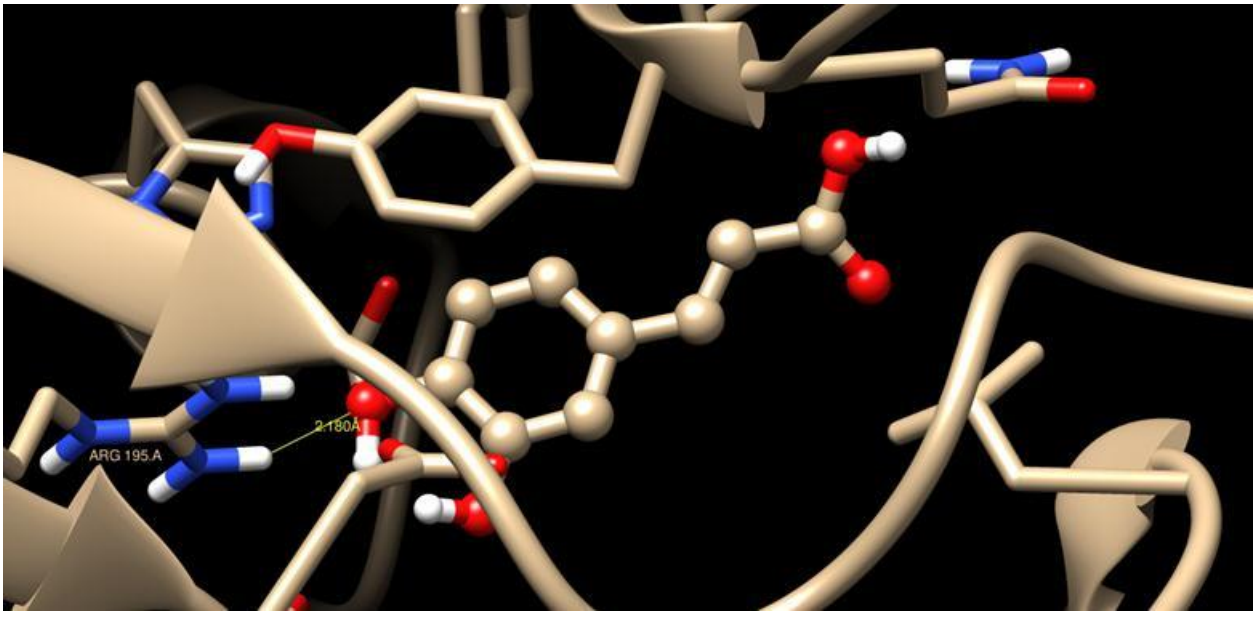

Figure 4B. The predicted structure details of hydrogen bondings of alpha-amylase residues with ligand caffeic acid

Table 2.The docking details of interacting alpha amylase amino acid residues with chlorogenic acid and caffeic acid as ligands

Ligands	Target PDB ID	Organism	Interacting amino acid residues	Binding energy (kcal/mol)	Bond length
Chlorogenic acid	1xcw	Homosapiens	ILE 235. A	-8.1	2.036 Å
			ASP 197.B		2.296 Å
			ASP 300 .A		2.646 Å
			HIS 305 .A		2.436 Å
Caffeic acid	1xcw	Homosapiens	ARG 195.A	-6.6	2.180 Å

The interaction occurs through formation of hydrogen bonding between the hydroxyl groups of the polyphenolsligands and the catalytic residues of the binding site which result in to the formation of bonding for stabilizing the interaction with the active site.

The docking analysis of glycogen phosphorelase (GP) amino acid residues formed eight hydrogen bonds between ARG 77.A, ARG 305.A and ARG 238.A amino acid residues and chlorogenic acid as shown in the Figure 5A, while caffeic acid formed two hydrogen bonds with GLN 67.A and ASP 223.A amino acid residues (Figure 5B).

The least binding energies for chlorogenic acid and caffeic acids are illustrated in the Table 3.

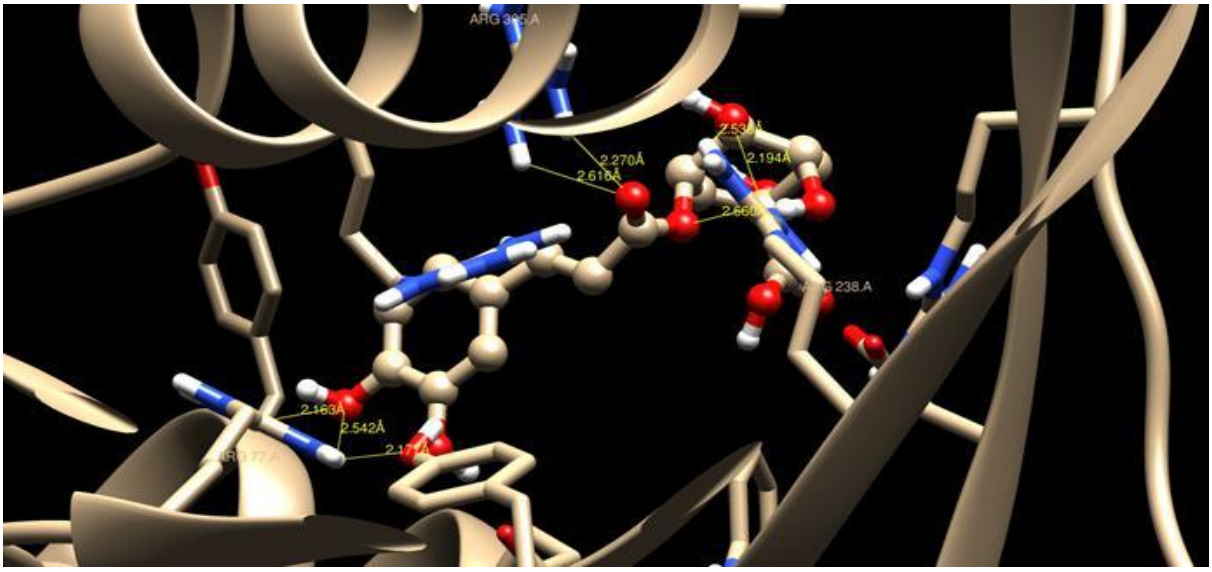

Figure 5A. The predicted structure details of hydrogen bonding of glycogen phosphorelase residues with ligand chlorogenic acid

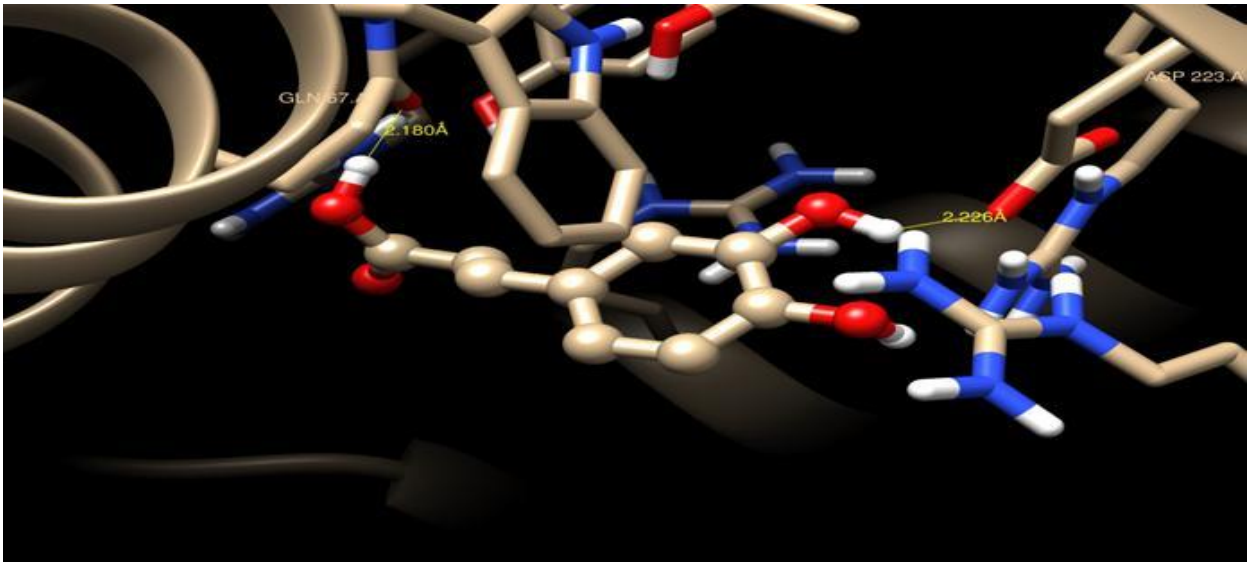

Figure 5B. The predicted structure details of hydrogen bonding of glycogen phosphorelase residues with ligand caffeic acid

Table 3.The docking details of interacting glycogen phosphorelase amino acid residues with chlorogenic acid and caffeic acid as ligands

Ligands	Target PDB ID	Organism	Interacting amino acid residues	Binding Energy (kcal/mol)	Bond length
Chlorogenic acid	1FA9	Homosapiens	ARG 77. A	-7.1	2.163 Å
			ARG 77. A		2.542 Å
			ARG 77.A		2.177 Å
			ARG 305.A		2.616 Å
			ARG 305.A		2.270 Å
			ARG 238.A		2.660 Å
			ARG 238.A		2.194 Å
			ARG 238.A		2.538 Å
Caffeic acid	1FA9	Homosapiens	GLN 67.A	-6.1	2.180 Å
			ASP 223.A		2.223 Å

Inhibition of hepatic glycogen phosphorylase is a promising strategy for treating hyperglycemia in diabetic patient. GP is important target for antidiabetic drugs which catalyzes the breakdown of glycogen to glucose-1-phosphate in liver and tissues. It has two forms, a and b, which are inter convertible. The b form is less active which is transformed into more active a form through phosphorelation.[20] The inhibition of glycogen phosphorelase inhibits the glycogenolysis pathway which in turn reduces the glucose production in liver and lower the glucose level in the blood. [21]

Hexokinase-I is another important enzyme which participate in glycolysis by catalyzing the phosphorelation of glucose into glucose-6-phosphate and provides sufficient energy for the glycolytic process to start. Hexokinase-I allows the muscle cells to take up glucose in the blood and use it as an energy source for different actions. [22]

Therefore the hexokinase was selected as a target to dock with polyphenols to see the binding affinity which may be helpful for the development of plant-based

pharmacophore as hexokinase activator. The docking studies of enzyme hexokinase I has shown a strong binding interaction via formation of three hydrogen bonding with chlorogenic acid (Figure 6A) and three hydrogen bonding with caffeic acid (Figure 6B).

The active binding sites of amino acid residues with bond length and binding energies are given in the Table 4.

The docking studies of chlorogenic acid and caffeic acid with three different target enzymes depicted that they are excellent molecules which docked effectively with the diabetes related targets proteins.

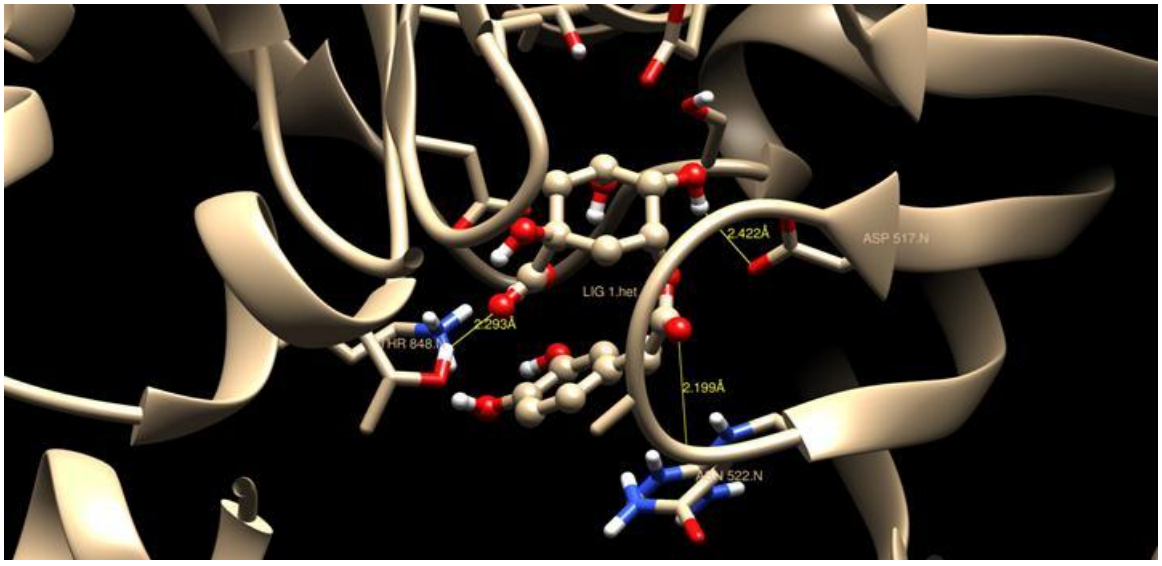

Figure 6A. The predicted structure details of hydrogen bonding of hexokinase-I residues with ligand chlorogenic acid

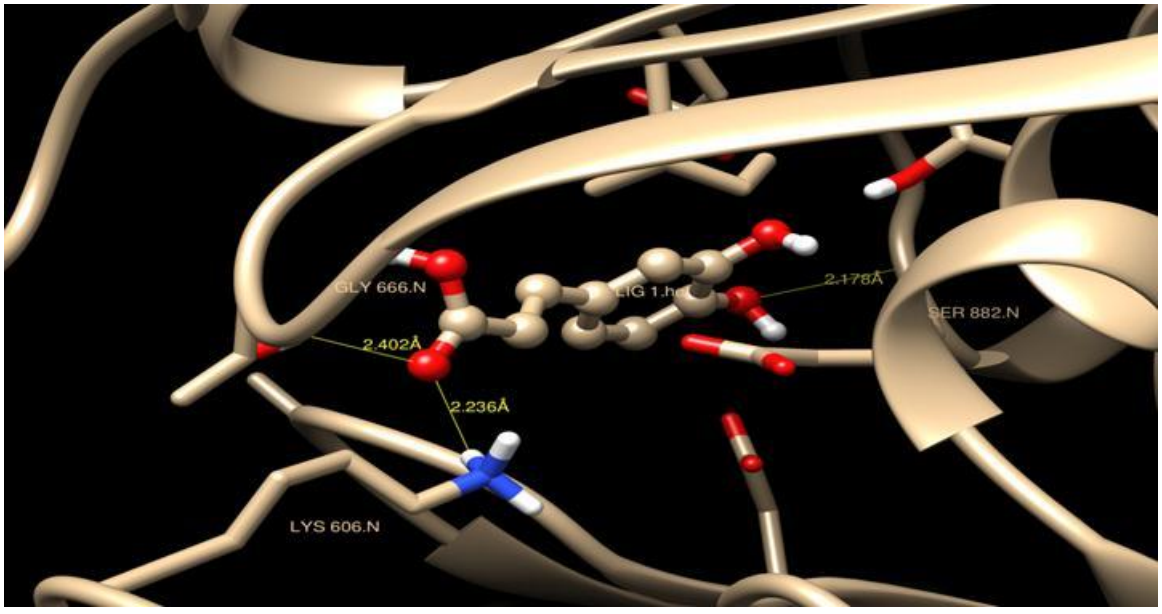

Figure 6B. The predicted structure details of hydrogen bonding of hexokinase-I residues with ligand caffeic acid

Table 4.The docking details of interacting hexokinase-I amino acid residues with chlorogenic acid and caffeic acid as ligands

Ligands	Target PDB ID	Organism	Interacting amino acid residues	Binding Energy (kcal/mol)	Bond length
Chlorogenic acid	1 dgk	Homosapiens	THR 848.N	-7.6	2.293 Å
			ASN 522.N		2.199 Å
			ASP 517.N		2.244 Å
Caffeic acid	1 dgk	Homosapiens	GLY 666.N	-6.4	2.402 Å
			LYS 606.N		2.236 Å
			SER 882.N		2.178 Å

Chlorogenic acid and caffeic acids can play a role in controlling diabetes and diabetes related complications by inhibiting the alpha amylase, glycogen phosphorelase and activating hexokinase.

MATERIALS AND METHODS

Un-ripe pod's collection and extraction

The un-ripe pods of the plant were harvested in the month of October from the Punjab University College of Pharmacy, University of the Punjab, Lahore, Pakistan and was identified by Dr. Zaheer-UD-Din (Government College University, Lahore, Pakistan) voucher number (GC. Herb. Bot. 2969) deposited at Botany Department, Government College University Lahore, Pakistan. The un-ripe pods were washed, dried under shade and pulverized. Powdered material (15 g) was extracted sequentially by maceration using 50 mL of solvents such as petroleum ether, chloroform and methanol. The material was macerated for 24 h with each of the solvents and procedure was repeated thrice. The extracts were filtered and dried in *vacuo*at 40^{o}C and termed as petroleum ether extract (PE), chloroform extract (CE) and methanol extract (ME).

Chemicals and solvents

The chemicals and solvents used in the present study included metronidazol (Siza International, Lahore, Pakistan), chlorogenic acid, alpha amylase and haemoglobine (China), acarbose (Bayer, Pakistan), methanol, acetonitrile, tetrahydrofuran (RCI Labscan, Thailand), caffeic acid, ferrulic acid, glucose, starch, chloroform, ethanol, acetone, sulphuric acid, FC reagent (Folin- Ciocalteau's), gallic acid, bovine serum albumin, enthrone reagent, nicotinic acid (Sigma Aldrich), n-hexane and acetic acid (E-Merck, Germany).

Software

The 1-Click Docking Mcule and UCSF Chimera 1.12 Software were used for docking and finding hydrogen-bonding affinities.

Antidiabetic activities

Alpha amylase inhibition activity

One milliliter enzyme solution (0.5 mg/mL, in 20 mM phosphate buffer of pH 6.9) and 1 mL of extract/standard (acarbose) having concentration 1 mg/mL were mixed and incubated at 37°C for 10 min. Afterwards, 1 mL of 1% starch solution was added in the reaction mixture and contents were again incubated at 37°C for 10 min. Finally, the reaction was stopped by adding 2 mL of dinitrosalycic acid and heating the mixture in boiling water bath for 8 min. After cooling the contents to room temperature, absorbance was noted at 540 nm [23] and % enzyme inhibition was calculated by the following formula:

$$Percentageactivity = [Absorbanceofcontrol - Absorbanceofsample] / Absorbanceofcontrol$$

Glucose uptake by yeast cells

The method described by Kumar et al. [24] was used to study glucose uptake by yeast cells. Briefly, the yeast cells were rinse with distilled water by centrifugation at 2500 rpm for 5min and procedure was repeated until supernatant

became clear. The yeast cell-pellet thus obtained was suspended in water to prepare suspension (10%, *V/V*). One milliliter of (5mM, 10mM and 20mM) glucose solution and 1mL of each extract/standard (1 mg/mL) were mixed and kept for incubation at 37°C for 10 min. Then, 100µL yeast suspension was mixed and mixture was further incubated for 1h at 37°C. Afterwards, the mixture was centrifuged at 2500 rpm for 5 min and supernatant was used to determine contents of glucose. Metronidazole was taken as a standard. The % of glucose uptake by yeast cells was determined using the following formula:

$$Percentageactivity = [Absorbanceofcontrol - Absorbanceofsample] / Absorbanceofcontrol$$

Non-enzymatic haemoglobin glycosylation inhibition activity

The sample/standard (gallic acid) and all the other solutions were made in phosphate buffer (0.01M) of pH 7.4. One milliliter solution of extract/standard (1 mg/mL), glucose (2%, *W/V*), haemoglobin (0.06%, *W/V*) and gentamycin (0.02%, *W/V*) were mixed and incubated in the dark at room temperature for 72 h. Then, the absorbance was measured at 440 nm.[25]The % inhibition of hemoglobin glycosylation was calculated using the following formula:

$$Percentageactivity = [Absorbanceofcontrol - Absorbanceofsample] / Absorbanceofcontrol$$

Statistical analysis

Statistical analysis of data was done by analyzing variance (ANOVA) and multiple comparison with Bonferoni test (SPSS 12.0). P values < 0.05 were considered significant.

Polyphenol-based characterization of ME by HPLC

The developed method [26] was used for the quantification of chlorogenic acid, caffeic acid and ferulic acid in the methanol extract of un-ripe pods of *Vincarosea*cv. Pink.

Preparation of standards and sample solutions

The stock solutions of chlorogenic, caffeic and ferrulic acids (1 mg/ml) were prepared in mobile phase (acetate buffer: methanol: acetonitrile: tetrahydrofuran in ratio of 65:20:10:5, *V/V/V/V*). Then a mixed standard solution was prepared by mixing 30, 15 and 15µL of stock solution of chlorogenic, caffeic and ferulic acids, respectively and volume was made 1 mL with the mobile phase. The sample solution was made by dissolving the extract in the mobile phase to get a solution having concentration of 5 mg/mL. The standards and sample was filtered using polytetrafluroethylene syringe filter of 0.45µm pore size (Whatmann, Maidstone, England).

System suitability parameters

The system suitability parameters such as number of theoretical plates (N), capacity factor (k′), tailing factor (As) and resolution (Rs) were calculated from the chromatogram obtained analyzing mixed standard solution of chlorogenic acid, caffeic acid and ferulic acid to ensure the accuracy and effectiveness of the system during the analyses.

Validation of HPLC method

The method was validated as per the ICH guidelines [27] for various parameters such as linearity, limit of detection, limit of quantification, precision, accuracy and robustness. For this purpose the different concentrations of chlorogenic, caffeic and ferulic acids (0.15, 0.3, 0.45, 0.9, 1.5, 2.4 and 3.0 µg/mL) were made in mobile phase. For determination of intraday accuracy and precision different concentrations for chlorogenic, caffeic and ferulic acids (0.15 to 0.45 µg/mL) were analyzed six times in the same day. For inter-day accuracy and precision, different concentrations of chlorogenic, caffeic and ferulic acids (0.15 to 0.45 µg/mL) were analysed once daily for six

consecutive days. The amount was calculated from the calibration curves, constructed on each day.

Chromatographic analysis

The analysis was performed by eluting the standards /sample (20 µL) with mobile phase at flow rate of 0.8 mL/min through the column (Agilant 5TC-C_{18} (250X4.6 mm) maintained at 30°C. The detection was carried out at 300 nm and peaks of the samples were identified with the standards. All the standards and sample were analysed in triplicate and contents of the standards in the sample were calculated from the calibration curve. The data acquisition was performed by LC/LCMS software from Windows.

Molecular docking studies

The docking studies of quantified chlorogenic and caffeic acids were performed with the target enzymes (pancreatic α-amylase, glycogen phosphorelase and hexokinase- I using 1-Click docking Mcule software and UCSF Chimera 1.12 Program. The smiles for each chlorogenic acid (C1C(C(C(CC1(C(=O)O)O) OC(=O)C=CC2=CC(=C(C=C2)O)O)O)O) and caffeic acid (C1=CC(=C(C=C1C=CC(=O)O)O)O) were noted from PUB-Chem database. The ligands were docked against the selected protein targets and docking score was noted. UCSF Chimera 1.12 Program was applied on PDB file of each ligands and hydrogen bonding with bond length was determined.

CONCLUSION

The un-ripe pods of *Vinca rosea* cv. Pink have shown significant antidiabetic activity. The active extract, methanol extract, contains chlorogenic acid and caffeic, which have shown good binding affinity with amino acids residues of alpha amylase, glycogen phosphorelase and hexokinase I.

ACKNOWLEDGMENTS

Authors are thankful to the University of the Punjab for promising research facilities.

REFERENCES

1. Stumvoll M, Goldstein BJ, Van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. *Lancet* 2005; 365: 1333-1346.
2. Issa R, Khattabi A, A.Alkaram T,Altamini O. The use of antidiabetic herbal remedies by Jordanian herbalist: A comparision of folkloric practice vs. evidence-based pharmacology. *Jordan Journal of Pharmaceutical Sciences*, 2019; 12: 3.
3. Ahmed MF, Kazim SM, Ghori SS., Mehjabeen, S.S., Ahmed, S.R., Ali, S.M., Ibrahim, M. Antidiabetic activity of *Vincarosea* extracts in alloxan-induced diabetic rats. *Int J Endocrinol*2010.
4. Nammi S, Boini KM, Lodagala, SD, Behara RBS. The juice of fresh leaves of *Catharanthusroseus* reduces blood glucose in normal and alloxan diabetic rabbits. *BMC Complement Altern Med* 2003; 3: 1-4.
5. Singh SN, Vats P, Suri S, Shyam R, Kumria MML, Ranganathan S, Sridharan K. Effect of an antidiabetic extract of *Catharanthusroseus* on enzymic activities in streptozotocininduced diabetic rats. *J Ethnopharmacol* 2001; 76: 269–277.
6. Mustafa NR, Verpoorte R. Phenolic compounds in Catharanthusroseus. *PhytochemRev* 2007; 6:243-258.
7. Rao SA, Ahmed FA. Simultaneous determination of phenolic compounds in *Catharanthusroseus* leaves by HPLC. *Int J Pharm Scis Res* 2014; 5: 977-981.
8. Wang Y, Huang L, Zhong YL. Effects of three kinds of dietary polyphenolyphenols on glucose and lipid metabolism in chemical-induced diabetic rats. *ActaNutrimentaSin* 2012; 34:572–575.
9. Ohnishi M, Matuo T, Tsuno T, Hosoda A, Nomura E, Taniguchi H, Sasaki H, Morishita, H. *Antioxidant activity and hypoglycemic effect of ferulic acid in STZ-induced diabetic mice and KK-Ay mice. Biofactors. 2004; 21: (1-4) 315-319.*
10. Jung UJ, Lee MK, Park YB, Jeon SM, Choi MS. Antihyperglycemic and antioxidant properties of Caffeic Acid in *db/db* Mice. *J Pharmacol Exp Ther* 2006; 318:476–483.
11. Clifford MN. Chlorogenic acids and other cinnamates-nature, occurrence and dietryburden. *J Sci Food Agric* 1999; 79:362-372.
12. Osguthorpe DJ, Sherman W, Haggler AT. Generation of receptor structural ensembles for virtual screening using binding site shape analysis and clustering. *Chem. Boil drug des*2012; 80: 182-193.
13. Lo-Piparo E, Scheib H, Frei N, Williamson G, Grigorov M, Chou CJ. Flavonoids for controlling starch digestion: structural requirements for inhibiting human α-amylase. *J Med Chem*2008; 51: 3555-3561.
14. Sales PMD, Souza PMD, Simeoni LA, Magalhaes PDO, Silveira D. α-amylaseinhibitors. A review of raw material and isolated compounds from plant source. *J Pharm Pharmaceut Sci* 2012; 15:141-183.
15. Daksha G, Chandrashekar KS, Pai G. *In vitro* antidiabetic activity of pentacyclictritrpenoids and fatty acid esters from *Bauhinia purpurea*. *Int J Pharmacol Pharm Technol* 2013; 2: 25-28.
16. Makita Z, Radoff S, Rayfield EJ, Yang Z, Skolnik E, Delaney V, Friedman EA, Cerami A, Vlassara H. Advanced glycosylation end products in patients with diabetic nephropathy. *N Engl J Med* 1991; 325: 836–842.
17. Yeh WJ, Hsia SM, Lee WH, Wu CH. Polyphenols with antiglycation activity and mechanisms of action: A review of recent findings. *J food drug anal* 2017;25: 84-92.
18. Adisa RA, Oke J, Olomu SA, Olorunsogo O. Inhibition of human haemoglobinglycosylation by flavonoid containing leaf extract of *Cnestisferruginea*. **j**. **Cameroon** Acad. *Sci.* 2004; 351-359.
19. Pereira DM, Valentao P, Sottomayor M, Federico F, Andrade PB. Phenolic compounds in *Catharanthusroseus*. *Nat Prod* 2013; 2093-2106.
20. *Nishibe S, Takenaka T, Fujikawa T, Yasukawa K, Takido M, Morimitsu Y, Hirota A, Kawamura T, Noro Y..*

Bioactive phenolic compounds from Catharanthusroseus and vincaminor. Nat Med 1996; 50: 378-383.

21. Baker, D. J., Timmons, J. A., Paul L. Greenhaff, P. L. Glycogen phosphorylase inhibition in type 2 diabetes therapy: A Systematic evaluation of metabolic and functional effect in rat skeletal muscle. *Diabetes* 2005; 54, 2453-2458.
22. Hayes, J. M., Kantsadi, A. K., Leonidas, D. Natural products and their derivatives as inhibitors of glycogen phosphorylase: Potential treatment for type-2 diabetes. *Phytochem Rev* 2014; 9360- 9366.
23. Aleshin, A., Bartunik, G., Bourenkov, G., Fromm, H., Hozatko, R., Kirby, C., Liu, X. Crystal structures of monomeric hexokinase type-I reveal ADP binding site and conformational changes relevant to allosteric regulation. *J MolBiol* 2000; 294: 1001-1015.
24. Ramakrishna SV, Suseela T, Ghilyan NP, Jalil A, Prema P, Lonsane BK, Ahmed SY. Recovery of amyloglucosidase from moulay bran. *Indian J Technol* 1982; 20: 476-480.
25. Kumar B, Dinesh A, Mitra M. In vitro and in vivo studies of antidiabetic Indian medicinal plants. A review. *J Herb Med Toxicol*2009; 3: 9-14
26. Parker KL, England JD, Da costa J, Hess RL, Goldstein DE. Improved colorimetric essay for glycosylated haemoglobin. *Clin Chem* 1981; 25: 669-672.
27. Javaid R. Determination of phenolic compounds in the methanol extract of *Syzygiumcumini*L. Unpublished M. Phil dissertation. University College of Pharmacy, University of the Punjab Lahore Pakistan. 2016.
28. Bansal S, De-Stefano A. Key elements of bioanalytical method validation for small molecules. *AAPS J* 2007; 9: 109-114.
29. SA Al-Awar M. Anti-diabetic activities of *Zizyphus spina-christi* seeds embryos extract on general characteristics of diabetes, carbohydrate metabolism enzymes and lipid profile in rats. *Jordan Journal of Pharmaceutical Sciences*, 2019; 12 (2).

النشاط المضاد لمرض السكر، والتوصيف القائم على مادة البوليفينول والتفاعل الجزيئي لمستخلص القرون غير الناضجة من فينكا الوردية صنف. لون القرنفل

عابدة قمر [1] *

[1] كلية الصيدلة، جامعة البنجاب، باكستان.

ملخص

هدفت الدراسة إلى التعرف على مستخلصات القرون غير الناضجة من نبات الفينكا الوردية. اللون الوردي للنشاط المضاد لمرض السكر والتوصيف القائم على البوليفينول للمستخلصات باستخدام HPLC. علاوة على ذلك ، تم استكشاف التفاعل الجزيئي للعلامات المحددة مع الأهداف المضادة لمرض السكر باستخدام برنامج الالتحام الجزيئي. تم تحضير وفحص مستخلصات مختلفة من القرون غير الناضجة باستخدام نماذج مضادة لمرض السكر مثل امتصاص الجلوكوز بواسطة خلايا الخميرة وتثبيط ألفا أميليز ومقايسات تثبيط الهيموجلوبين بالجليكوزيل. يتميز المستخلص الأكثر نشاطًا باستخدام HPLC باستخدام أحماض الكلوروجينيك والكافيين والفيروليك كواسمات تحليلية. تم أخذ العلامات المحددة كروابط للالتحام الجزيئي مع α-amylase و glycogen phosphorelase و hexokinase–باستخدام 1− Click Docking Mcule Software، وإيجاد روابط ارتباط الهيدروجين بواسطة UCSF Chimera 1.12. أظهرت مستخلصات الميثانول نشاطًا مضادًا لمرض السكر أعلى بنسبة 37.77 و 71.16 و 53.52٪ في تثبيط مقايسة α-amylase وزيادة امتصاص الجلوكوز بواسطة خلايا الخميرة وتثبيط Hb-glycosylation على التوالي. تم العثور على المستخلص يحتوي على 0.25 مجم / جرام من الكلوروجينيك و 0.11 مجم / جرام من أحماض الكافيين. تم العثور على هذه العلامات لتكون روابط جيدة للأهداف المتعلقة بمرض السكري. تشير النتائج إلى أن مستخلص الميثانول له نشاط مضاد لمرض السكر، والذي يمكن تخصيصه لأحماض الكلوروجينيك والكافيين. يمكن استخدام هذه المركبات كواسمات دوائية لتوحيد المستخلصات.

الكلمات الدالة: فينكا الوردية السيرة الذاتية. الوردي، النشاط المضاد لمرض السكر، القرون غير الناضجة، البوليفينول، HPLC عكسي الطور، التفاعل الجزيئي.

* ***المؤلف المراسل: عابدة قمر***

abidabilal23@gmail.com

تاريخ استلام البحث 2021/3/10 وتاريخ قبوله للنشر 2021/11/12.