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CDK1 AND CDK2 AS POTENTIAL TARGETS FOR ANTICANCER ACTIVITY

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Abstract

Cancer is one of the major causes of death throughout the world. The treatment for this disease is done using several therapies such as chemotherapy, radiation therapy, immunotherapy, hormone therapy or targeted therapy. Targeted therapy works by targeting the cancer specific proteins or genes. Cyclin dependent-kinases *i.e.*, CDKs are the enzymes (proteins) that control the cell cycle. The CDKs work together with their regulatory partners- the cyclins. Thus CDK-cyclin complexes are central regulators of cell cycle progression. Plants have a large number of bioactive metabolites or phytochemicals and many of these phytochemicals possess potential anticancerous activity. In this experiment CDK1 and CDK2 are taken as the protein targets and 37 natural ligands of the plant *Thevetia peruviana* (Pers.) K. Schum have been docked with them for finding the best phytochemical possessing anticancer activity.

Key words : CDK1, CDK2, natural ligands, molecular docking.

Introduction

Cancer is one of the principal causes of death worldwide and for the treatment of this disease several attempts are made by the researchers across the world. (Magalhaes *et al*., 2018). Among the several noteworthy advances that have been made, targeted therapies are considered as the most significant one. The cell cycle is an important source for target identification. It helps in understanding fundamental pathways of cancers to facilitate new opportunities to discover new targets for cancer therapy (Mathews *et al*., 2010; Moen *et al*., 2007). The cell cycle consists of two distinct phases- mitosis and interphase. In mitosis (M) phase the cell undergoes cell division. The interphase comprises of pre-DNA synthesis (G1) phase, DNA synthesis (S) phase and pre-division (G2) phase. Following interphase, the cell returns to the quiescence (G0) phase. G1 phase is the first step in cell cycle progression. In S phase DNA content changes from 2N to 4N. When the chromosomes are correctly duplicated cells can enter G2 to prepare for the M phase. There are two regulatory processes which primarily control cell cycle progression. The first one is the phosphorylation of specific proteins by cyclin-dependent kinases (CDKs) and their dephosphorylation by phosphatase. The second one is specific proteolytic degradation by the ubiquitin-proteasome system. These regulatory mechanisms ensure that in G1 phase the cells which are experiencing DNA damage do not enter the S phase. In this way the chromosomes are correctly replicated before they segregate into the daughter cells (Bai *et al*., 2017).

Each stage in the cell cycle is securely regulated by CDKs belonging to a well conserved family of threonine/serine protein kinases. The CDKs are master regulators of the cell division cycle in association with the cyclin regulatory subunits (Ruijtenberg and Heuvel, 2016). CDK-cyclin complexes are central regulators of cell cycle progression as they transduce extracellular cues, such as growth factor signals and the presence of nutrients in the cell (Lapenna *et al*., 2009). Till todays date 20 different CDKs have been reported in mammalian cells. The cyclins are also about the same in number as the CDKs (Floquet *et al*., 2015). Among the CDK family CDK7, CDK8 and CDK9 regulate transcription while CDK1, CDK2, CDK4 and CDK6 promote cell cycle progression (Asghar *et al*., 2015). Different phases of cell cycle require different cyclins. Cyclin D1, D2 and D3 are associated with CDK4 and CDK6. They are essential

for regulating various events in early G1 phase (Mikhail *et al*., 2015). In G1 phase cyclin E associates with CDK2 for regulating late G1 phase and induction of DNA synthesis in early S phase. For G1/S phase transition the cyclin E/CDK2 complex is extremely important. As the cell cycle progresses, cyclin A replaces cyclin E as the companion of CDK2 and then controls DNA synthesis and replication in the S phase. Further cyclin A associates with CDK1 to promote entry into the M phase. CDK1 cooperates with other kinases to drive the transition from G2 to M phase thus contributing to mitotic progression in cell division. Cyclin B replaces cyclin A and the cyclin B/CDK1 complex activates mitosis (Bai *et al*., 2017). CDKs emerged as potential molecular targets for cancer therapy approximately 20 years ago (Asghar *et al.*, 2015). The imbalance in production of cyclins or production at improper time leads to abnormality and mutation in the cell cycle, resulting in aberrant growth and unlimited proliferation, as seen in cancerous cells (Morgan, 2007; Rastogi *et al*., 2013). Medicinal plants have a large number of bioactive metabolites (phytochemicals). These metabolites derived from medicinal plants in apt doses and in suitable form could be an important approach for the prevention and treatment of cancer (Ortega and Campos, 2019).

Materials and Methods

Selection of Ligand and Ligand preparation

A total of 37 natural ligands (phytochemicals) of the plant *Thevetia peruviana* (Pers.) K. Schum were selected (Goutam and Goutam, 2006). They were cleaned using Marvin suite. Marvin suite is a desktop toolkit. It helps in drawing, editing, publishing, importing and exporting chemical structures. After the cleaning process the ligands were further converted into 2D structures.

Selection of target

Protein target selection was done using PDB (Protein data bank) and literature review. 2 target proteins were selected Cdk1 and Cdk2.

Target Ligand Docking using YASARA structure

YASARA is considered as one of the best software for docking. The CDK1 (5LQF) and CDK2 (1E1V) were chosen as the receptor. Prior to docking it had been ensured that the water molecules were removed and the protein targets were completely cleaned. It had been also ensured that the energy was minimized prior to docking.

Results and Discussion

Protein-Ligand docking

Docking of protein CDK1 (5LQF) and CDK2 (1E1V) was carried out with 37 natural ligands using YASARA structure. The post-processing of docking resulted in top 5 Phytochemicals that were ranked on the basis of their binding energy (tables 1 and 2).

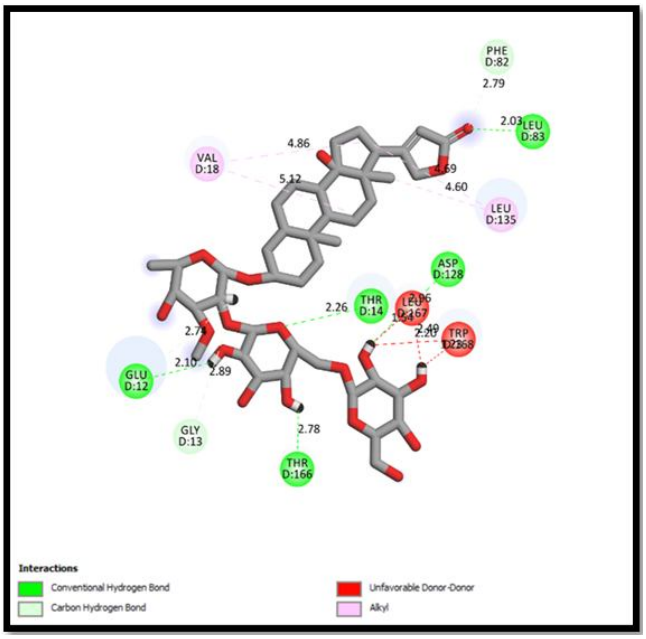

Fig. 1 : 2D representation of 5LQF complexed with Cerbroside.

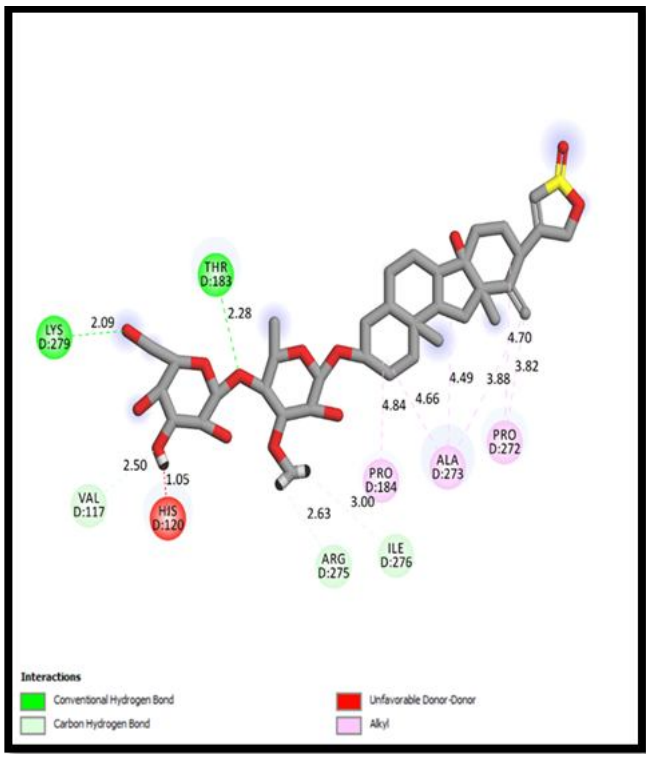

Fig. 2 : 2D representation of 5LQF complexed with Thevetioside D.

Discussion

The result of docking with the natural ligands of the plant *Thevetia peruviana* (Pers.) K. Schum such as

Table 1 : Docking Results of the top 5 phytochemicals showing interaction residues with receptor.

S. no.	Ligand Name	Binding energy [kcal/mol]	Number of hydrogen bonds	Contacting receptor residues
1	Cerbroside	8.984	7	Ile 10, Gly 11, Glu 12, Gly 13, Thr 14, Tyr 15, Val 18, Ala 31, Phe 82, Leu 83, Asp 86, Asp 128, Lys 130, Gln 132, Asn 133, Leu 135, Ala 145, Asp 146, Leu 149, Arg 158, Thr 166, Leu 167, Trp 168, Tyr 169
2	Thevetioside D	8.963	5	Ile 116, Val 117, His 120, Ser 121, Glu 173, Ser 178, Arg 180, Tyr 181, Ser 182, Thr 183, Pro 184, Ile 187, Trp 228, Pro 229, Glu 230, Val 231, Leu 234, Asp 271, Pro 272, Ala 273, Arg 275, Ile 276, Ser 277, Gly 278, Lys 279
3	Thevetioside E	8.909	6	Ile 116, Val 117, His 120, Ser 121, Glu 173, Ser 178, Arg 180, Tyr 181, Ser 182, Thr 183, Pro 184, Ile 187, Trp 228, Pro 229, Glu 230, Val 231, Leu 234, Asp 271, Pro 272, Ala 273, Arg 275, Ile 276, Ser 277, Gly 278, Lys 279, Met 280
4	Quercetin35 digalactoside	8.909	6	Glu 8, Lys 9, Ile 10, Gly 11, Glu 12, Gly 13, Val 18, Ala 31, Met 32, Lys 33, Val 64, Phe 80, Phe 82, Leu 83, Ser 84, Met 85, Asp 86, Lys 89, Lys 130, Gln 132, Asn 133, Leu 135, Ala 145, Asp 146, Leu 149
5	Cerberoside	8.827	10	Ile 10, Gly 11, Glu 12, Gly 13, Thr 14, Tyr 15, Val 18, Ala 31, Lys 33, Asp 86, Lys 88, Asp 128, Lys 130, Gln 132, Asn 133, Leu 135, Asp 146, Leu 149, Arg 158, Thr 166, Leu 167, Trp 168

Table 2 : Docking Results of the top 5 phytochemicals showing interaction residues with receptor.

S. no.	Ligand Name	Binding energy [kcal/mol]	Number of hydrogen bonds	Contacting receptor residues
1	Kaempferol	8.861	3	Ile 10, Gly 11, Glu 12, Gly 13, Thr 14, Val 18, Ala 31, Lys 33, Phe 82, Leu 83, Gln 85, Asp 86, Lys 129, Gln 131, Asn 132, Leu 134, Asp145
2	Quercetin	8.632	5	Ile 10, Gly 11, Glu 12, Gly 13, Thr 14, Val 18, Ala 31, Lys 33, Phe 82, Leu 83, His 84, Gln 85, Asp 86, Lys 89, Lys 129, Gln 131, Asn 132, Leu 134, Ala 144, Asp 145
3	Thevetioside F	8.547	10	Arg 122,, Leu 124, Arg 126, Ala 149, Arg 150, Gly 153, Val 154, Arg 169, Ile 173, Gly 176, Cys 177, Lys 178, Tyr 179, Tyr 180, Ser 181, Thr 182, Glu 208, Asp 235
4	Thevetioside H	8.547	10	Arg 122, Leu 124, Arg 126, Ala 149, Arg 150, Gly 153, Val 154, Arg 169, Ile 173, Gly 176, Cys 177, Lys 178, Tyr 179, Tyr 180, Ser 181, Thr 182, Glu 208, Asp 235
5	Thevetin C	8.525	6	Ala 95, Leu 96, Thr 198, Arg 199, Arg 200, Leu 202, Phe 203, Pro 204, Arg 214, Arg 217, Thr 218, Trp 243, Ala 244, Gln 246, Lys 250, Val 251, Val 252, Pro 253, Pro 254

Cerbroside displays 7 hydrogen bonds. Other ligands such as Thevetioside D displays 5 hydrogen bonds, following Thevetioside E which displays 6 hydrogen bonds. Quercetin 3,5-digalactoside displays 6 hydrogen bonds and Cerberoside displays 10 hydrogen bonds. The result of docking with the natural ligand Kaempferol displays 3 hydrogen bonds. Other ligands of the same plant such as Quercetin displays 5 hydrogen bonds, following Thevetioside F which displays 10 hydrogen bonds. Thevetioside H displays 10 hydrogen bonds and Thevetin C displays 6 hydrogen bonds.

The binding energy of the natural ligands of the plant *Thevetia peruviana* (Pers.) K. Schum such as Cerbroside, Thevetioside D, Thevetioside E, Quercetin 3,5-digalactoside and Cerberoside are 8.984, 8.963, 8.909, 8.909 and 8.827, respectively. The binding energy of the

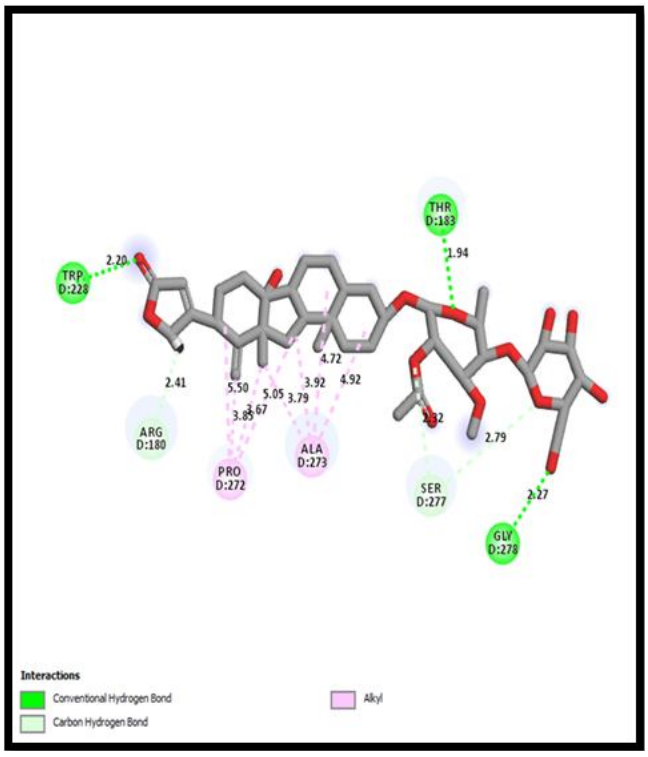

Fig. 3 : 2D representation of 5LQF complexed with Thevetioside E.

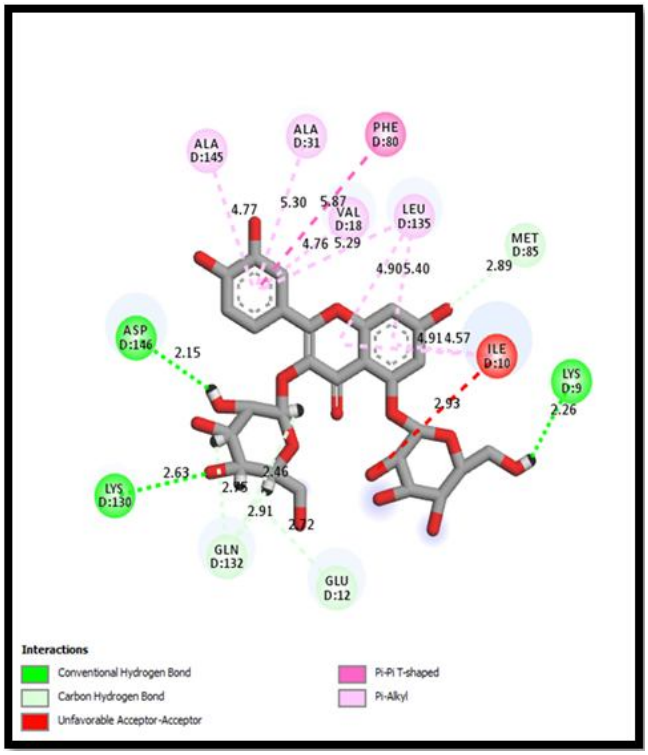

Fig. 4 : 2D representation of 5LQF complexed with Quercetin.

natural ligands of the plant such as Kaempferol, Quercetin, Thevetioside F, Thevetioside H and Thevetin C are 8.861, 8.632, 8.547, 8.547 and 8.525, respectively.

Bioactive extracts or metabolites derived from

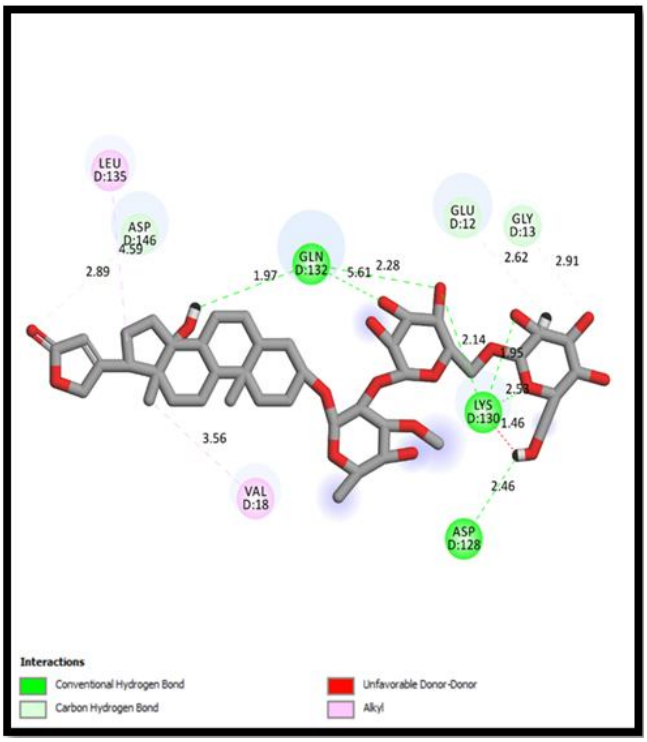

Fig. 5 : 2D representation of 5LQF complexed with cerebroside.

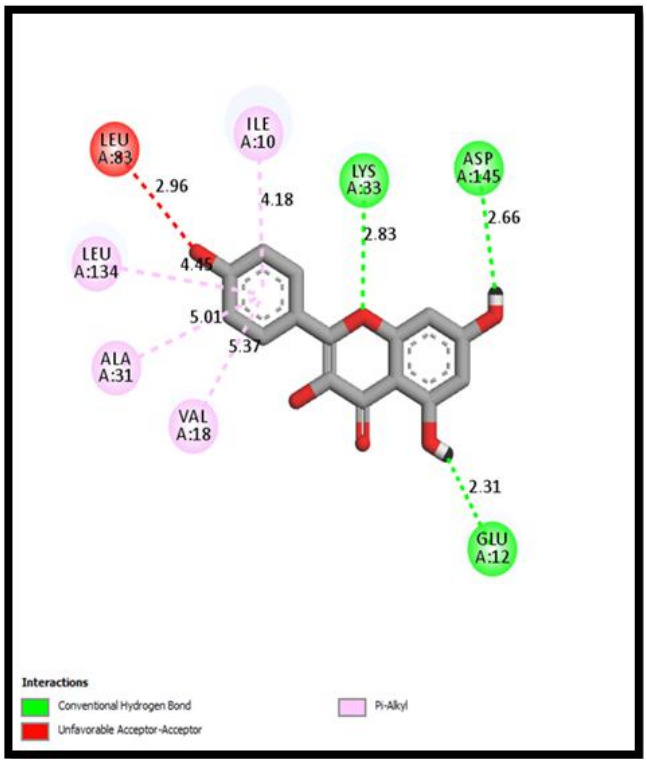

Fig. 6 : 2D representation of 1E1V complexed with Kaempferol.

medicinal plants have immense potential to prevent and treat cancer. Bioactive compounds like Curcumin, Zingerol, Nimbolide have proved to possess anticancer properties (Hejazi *et al*., 2013; Al-Asmari *et al*., 2015; Elumalai and Arunakaran, 2014). In the current experiment, the

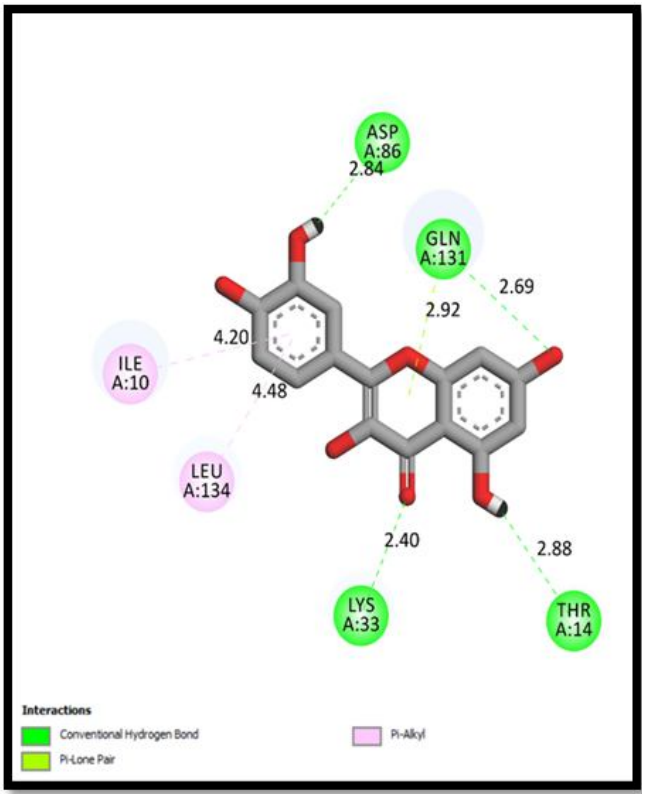

Fig. 7 : 2D representation of 1E1V complexed with Quercetin.

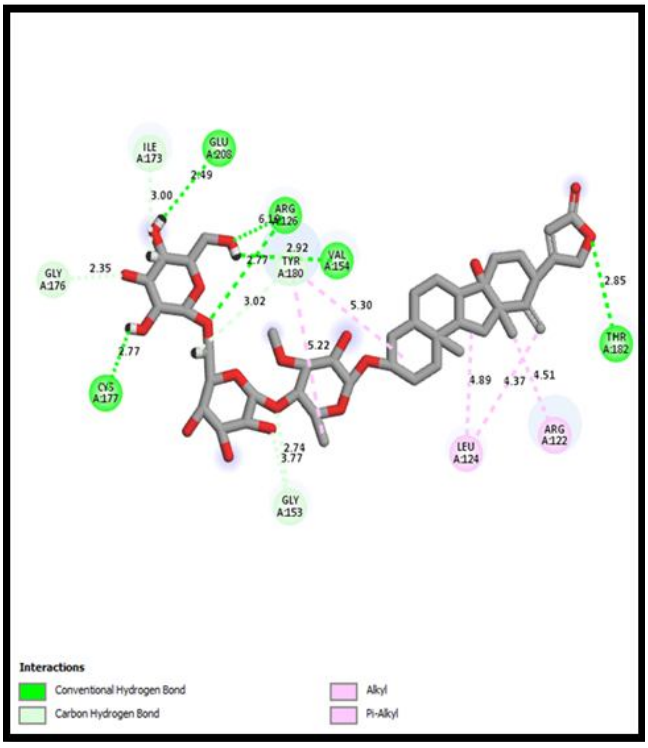

Fig. 8 : 2D representation of 1E1V complexed with Thevetioside F.

bioactive compounds Kaempferol and Quercetin have exhibited the best result in comparison to the other phytochemicals. On Comparing the number of Hydrogen

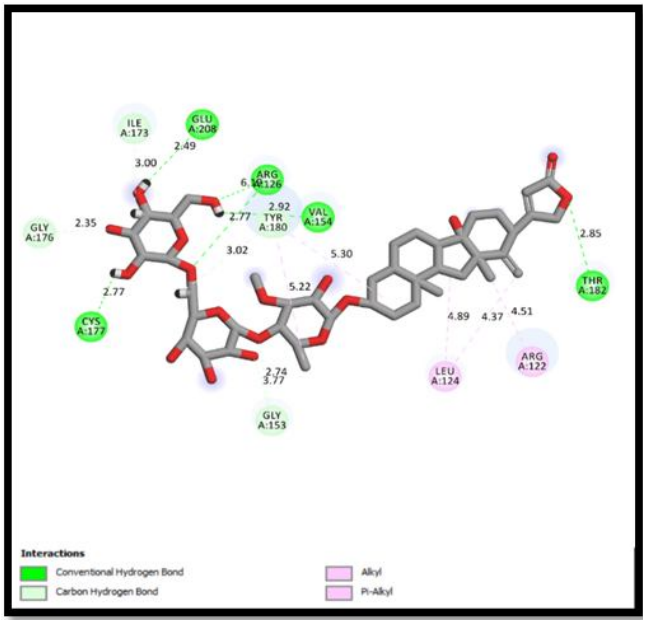

Fig. 9 : 2D representation of 1E1V complexed with Thevetioside H.

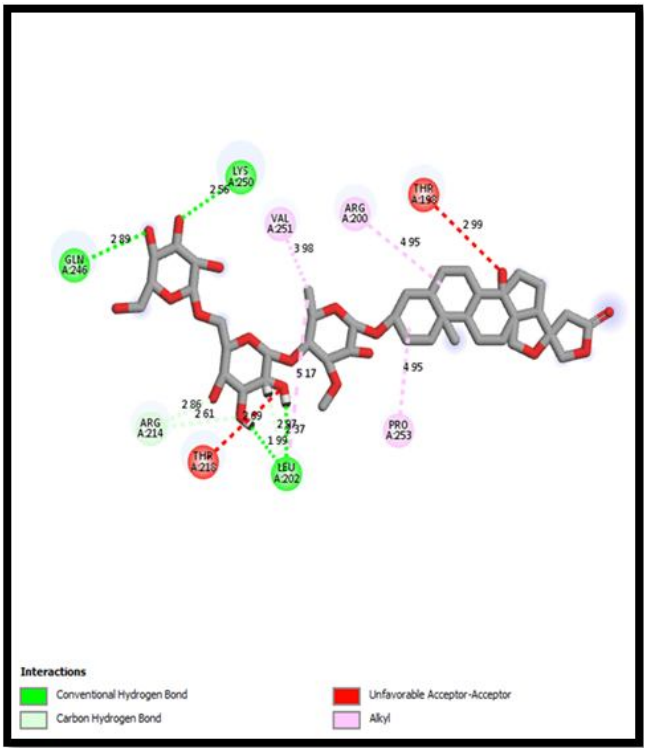

Fig. 10 : 2D representation of 1E1V complexed with Thevetin C.

bonds and the binding energy it can be concluded that the result of docking of the protein CDK2 is better than CDK1. Among all the CDKs, CDK2 is known to be an important kinase in tumorigenesis and proliferation in many cancer types including lung cancer, liver cancer, colon cancer, prostate cancer, ovarian cancer, cholangiocarcinoma, oral squamous cell carcinoma and breast cancer (Opyrchal *et al*., 2014; Simak *et al*., 2009;

Flores *et al*., 2010; Wingren *et al*., 2018; Huang *et al*., 2014; Corsino *et al*., 2008; Mihara *et al*., 2001; Zheng, *et al*., 2016; Shi *et al*., 2015). There is a strong evidence showing that CDK2 is functionally linked with hyper proliferation in multiple cancer cells and is a potential therapeutic target for Cancer therapy (Chohan *et al*., 2015). Previously in certain studies of protein-ligand docking the protein targets CDK1 and CDK2 had been chosen as the targets for natural compounds such as Kaempferol, Quercetin, Luteolin, Apigenin, Genistein, Daidzein, Naringenin, Hesperetin, Taxifolin, Catechin and Curcumin (Casagrande *et al*., 2001; Huang *et al*., 2014). In our in-silico experiment 2 major phytochemicals viz. Kaempferol and Quercetin have been found promising.

Conclusion

Cyclin dependent kinases are the favorable targets for anticancer drugs, as they are involved in cell cycle events like progression, control, transcription and DNA repair. Understanding the role and pathways involved in the cell cycle, CDKs provide informative knowledge of them for being effective target for cancer therapy (Ghorbani and Karimi, 2015). The phytochemicals such as Kaempferol, Quercetin, Thevetioside F, Thevetioside H, Thevetin C, Cerbroside, Thevetioside D, Thevetioside E, Quercetin 3,5-digalactoside and Cerberoside could be helpful in further targeting the protein targets CDK1 AND CDK2. These *in silico* studies through virtual screening can be helpful in shortlisting the promising phytochemical candidates prior to the *in vitro* anti-cancer studies.

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