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CISSAMPELOS PAREIRA: PROTEIN-LIGAND DOCKING TO IDENTIFY SUITABLE TARGETS FOR HEPATOCELLULAR CARCINOMA (HCC) BY *IN-SILICO* TECHNIQUES AND TOOLS

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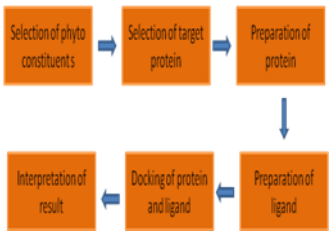
Graphical abstract



Abstract

Protein-ligand interaction plays a major role in identification of the possible mechanism by which a ligand can bind with the target and exerts the pharmacological action. The present study aims to identify new possible candidates for treating Hepatocellular Carcinoma (HCC) by docking the reported phytochemicals present in *Cissampelos pareira* with the well known HCC targets using *in-silico* techniques. Although *C. pareira* demonstrated *in vitro* and *in vivo* anti-heptatocellular carcinoma activities, the mechanism remains uncertain. Selected compounds from *C. pareira* were docked using GLIDE software with known targets of hepatocellular carcinoma *viz.* Aurora Kinase, c-Kit, Fibroblast Growth Factor (FGF), Nuclear Factor kappa B (NF-κB), B-cell lymphoma-extra large (Bcl-xL) and Vascular Endothelial Growth Factor (VEGF). Among the compounds docked, pareitropone and pareirubrine B exhibited good hydrogen bonding interactions and binding energy with the targets of HCC taken in the study. Hence these compounds deserve consideration for further studies towards HCC.

Keywords: *Cissampelos pareira,* GLIDE, docking

Abstrak

Interaksi protein-ligan memainkan peranan utama dalam mengenalpasti mekanisme yang mungkin di mana sesuatu ligan dapat bergabung dengan sasaran dan mendorong tindakan farmakologi. Kajian ini bertujuan untuk mengenalpasti calon baharu untuk merawat karsinoma hepatoselular (HCC) melalui gabungan dengan sebatian fitokimia yang dilaporkan terkandung dalam tumbuhan *Cissampelos pareira* dengan sasaran HCC menggunakan teknik *in-siliko*. Meski pun *C. pareira* didapati mempunyai aktiviti anti-karsinoma heptatoselular secara *in vitro* dan *in vivo*, mekanismenya masih tidak dapat dipastikani. Sebatian terpilih daripada *C. pareira* yang tergabung menggunakan perisian GLIDE dengan sasaran karsinoma hepatoselular seperti Kinase Aurora, c-Kit, Faktor Pertumbuhan Fibroblast (FGF), Faktor Nuklear kappa B (NF-κB), Sel-B limfoma-Lebih Besar (Bcl-xL) dan Faktor Vaskular Pertumbuhan Endotelial (VEGF) adalah dikaji. Antara sebatian yang digabung, pareitropona dan pareirubrina B menunjukkan interaksi ikatan hidrogen yang baik dengan ikatan tenaga sasaran HCC yang dikaji. Justeru sebatian ini layak dipertimbangkan dengan lebih lanjut dalam kajian terhadap HCC.

Kata kunci: *Cissampelospareira,* GLIDE, gabungan

1.0 INTRODUCTION

Hepatocellular carcinoma, the third common cancer, accounts for more than 5,98,000 new cases per year worldwide [1]. The present study aimed to perform docking of ligands which were reported in the plant *Cissampelos pareira* with the macromolecular targets which involves in various apoptosis cell signaling pathways [2]. Our lab findings indicate that this plant have *in vitro* cytotoxic activity in MCF-7, Hep2 and Hela cancer cell line and *in vivo* anticancer activity [3,4]. In order to find out the best candidate of the plant constituents, the reported phyto-constituents of *Cissampelos pareira* were docked with the various targets responsible for different signaling pathways like Aurora kinase, proto-oncogene c-Kit (c-Kit), B-cell lymphoma-extra large (Bcl-xL), Nuclear Factor kappa B (NF-κB), Fibroblast Growth Factor (FGF) and Vascular Endothelial Growth Factor (VEGF).

Plants are rich in secondary metabolites like alkaloids, glycosides, tannins, terpenoids, flavanoids etc. *Cissampelo spariera* Linn. (Menispermaceae) is a climbing shrub that grows in topical and sub topical India. *Cissampelos pareira* is also called as Midwives's herb as it is used in treating female reproductive system. It is used as astringent, antispasmodics, analgesic, antipyretic, diuretic, antilithic and emmenagogue [5]. It is reported to have cardio protective [6], hepatoprotective [7], *in vitro* antioxidant and immunomodulator [8], antifertility [9], antiarthritic [10] and anti-inflammatory activities [11]. The plant is reported to have phytoconsituents like cissamine (**1**), cissamperine (**2**), cycleanine (**3**), dicentrine (**4**), grandirubrine (**5**), hayatinine (**6**), insularine (**7**), isochondrodendrine (**8**), l-curine (**9**), pareirubrine A (**10**), pareirubrine B (**11**) and pareitropone (**12**) (Figure1) [12-16].

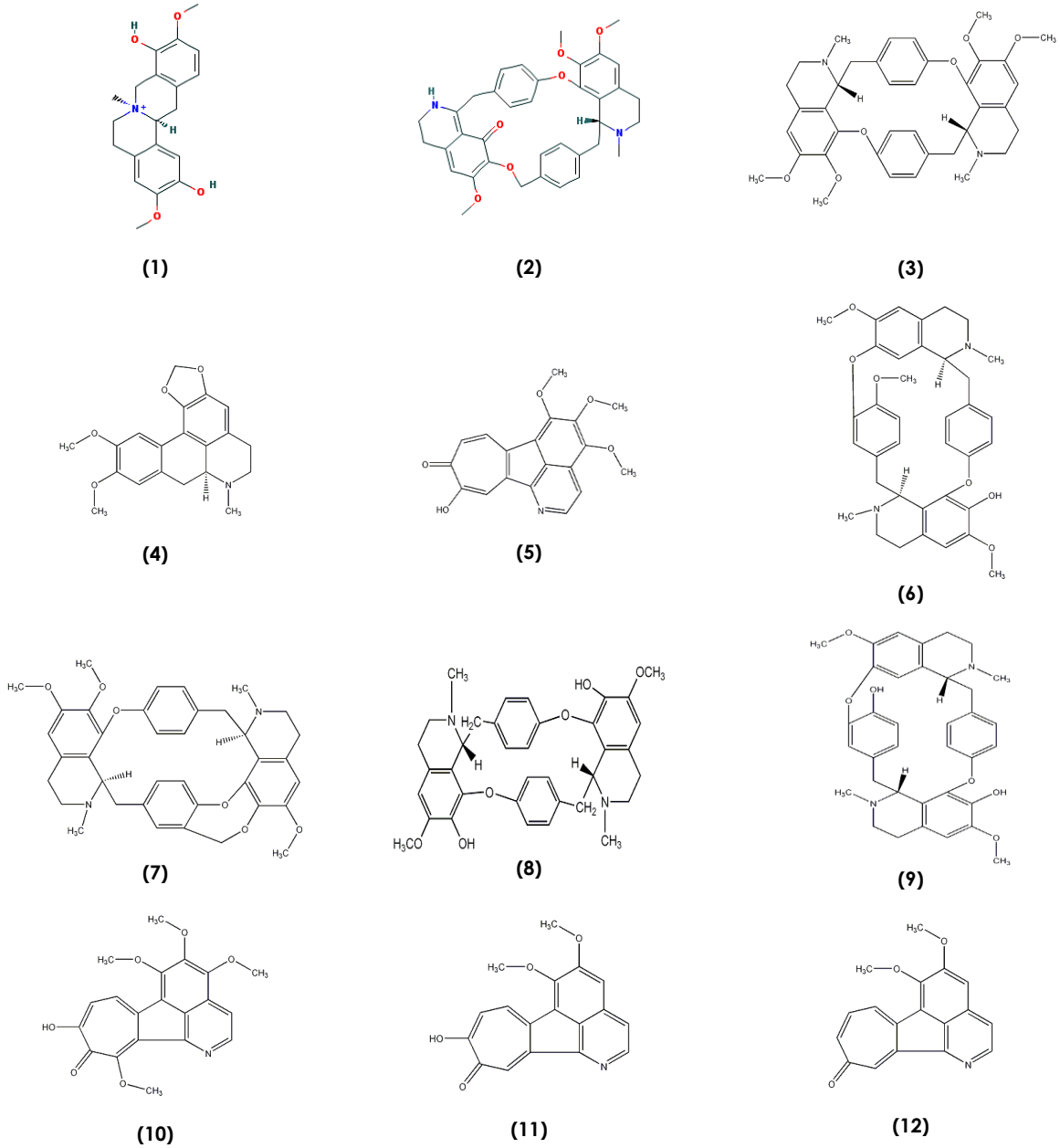


Figure 1 Chemical compounds of *C. pareria* taken for docking

2.0 EXPERIMENTAL

2.1 Ligand Preparation

A set of quinoline alkaloids reported in *Cissampelos pareira* were used for the present docking studies to select the best suitable targets for hepatocellular carcinoma by *in-silico* method. All the ligands were built using Maestro build panel. LigPrep is a utility of Schrödinger software that generates 3D structures from 2D representation. LigPrep also assigns an appropriate bond order with correct chiralities for each successfully processed input structure and produce a number of structures from each input structure with various ionization states, tautomers, stereoisomers and ring conformations. Subsequently, the structures were optimized by means of OPLS-2005 using a default setting in the LigPrep. The collected ligands were prepared by using LigPrep [Schrondinger] which uses MMFF 94s force field and gave the corresponding low energy 3D conformers of the ligands.

2.2 Protein Preparation

LigPrep is a utility of Schrödinger software that generates 3D structures from 2D representation. LigPrep also assigns an appropriate bond order with correct chiralities for each successfully processed input structure and produce a number of structures from each input structure with various ionization states, tautomers, stereoisomers and ring conformations. Before docking the ligands into the protein active site, the protein was prepared using protein preparation wizard of Schrondinger's molecular docking software. In this protein preparation, all water molecules and hetero atoms were removed. The screened ligands were then docked into the prepared grid, for which "standard precision mode" was selected. No constraints were defined.

2.3 Grid Generation and Ligand Docking

Docking was carried out using GLIDE (Grid-Based Ligand Docking with Energies) software [17]. GLIDE searches for favorable interaction between one or more ligand molecules and a receptor molecule. Grids were defined by centering them around the ligand in the crystal structure using the default box size setting in Glide: scaling of van der Waals radii of protein atoms partial atomic charge of less then 0.25 by 1.0. Hydrogen bond constraints were not applied. Flips of 5- and 6-member rings were allowed, and non-planar conformation of amide bonds was penalized. Van der Waals radii of ligand atoms with partial atomic charge less than 0.15 was scaled by 0.8. The prepared ligands were docked against the Aurora Kinase, c-Kit, FGF, NF-κB, Bcl-xL and VEGF receptors. All docking calculations were performed using the "Extra precision" (XP) mode of Glide program (Glide, 2009). Glide uses a hierarchical series of filters to search for possible locations of the ligand in the active-site region of the receptor. The initial filters test the spatial fit of the ligand to the defined active site, and examine the complementarity of ligand–receptor interactions using a grid-based method. Poses that pass these initial screens enter the final stage of the algorithm, which involves evaluation and minimization of a grid approximation to the OPLS-AA nonbonded ligand–receptor interaction energy. Final scoring is then carried out on the energy-minimized poses. The minimized poses are rescored using Schrödinger's proprietary GlideScore (GScore) scoring function. GScore is a modified version of ChemScore, but includes a steric-clash term and adds buried polar terms devised by Schrödinger to penalize electrostatic mismatches.

$$GScore = a \times vdW + b \times Coul + Lipo + Hbond + Metal + BuryP + RotB + Site$$

Where
vdW—Van der Waal energy, Coul—Coulomb energy, Lipo—Lipophilic contact term, bond—Hydrogen-bonding term, Metal—Metal-binding term, BuryP—Penalty for buried polar groups, RotB—Penalty for freezing rotatable bonds, Site—Polar interactions at the active site; and the coefficients of vdW and Coul are: = 0.065, b = 0.130. All docking computations were carried out with the Linux OS (Red Hat Enterprise WS 5.0).

3.0 RESULTS AND DISCUSSION

The binding of various phytoconsitutents from *Cissampelos pareira* against different targets and their G-score, glide energy, hydrogen interactions and their bond distance has been discussed below.

Binding Mode with Aurora Kinase Protein Target

The binding efficiency of the phytoconstituents from *Cissampelos pareira* against the target Aurora kinase is shown in Table 1 and the G-score value ranges from -8.355 to -2.634. All tested compounds showed good glide score and among that pareitropone (**12**), dicentrine (**4**) and pareirubrine B (**11**) showed marked interactions with the G-score value of -8.355, -8.242, -7.241 and glide energy of -57.650, -56.106, -56.352 respectively. The various hydrogen bonding interactions that bind the constituents of *Cissampelos pareira* with the Aurora kinase target are LEU (139), ALA (213), PRO (214), GLU (260), LYS (143), TYR (212), LEU (139), ALA (213), LEU (139), LEU (139), GLY (142), ALA (213), ALA (213), HIS (64) and ARG (56).

Binding Mode with C-Kit

The binding efficiency of the phytoconstituents from *Cissampelos pareira* against the target C-kit are shown in Table 2 and the G-score value ranges from -7.318 to -3.012. Out of 13 tested compounds, 11 compounds showed good glide score and among that cissamine (**1**), pareirubrine B (**11**) and

grandirubrine (**5**) showed marked interactions with the G-score value of -7.318, -7.305, -7.238 and glide energy of -54.064, -53.594, -56.052 respectively. The various hydrogen bonding interactions that bind the constituents of *Cissampelos pareira* with the C-kit target are CYS (674), CYS (673), LYS (593), LEU (595),

ASN (680), CYS (673), CYS (673), TYR (672), LYS (593), and CYS (673).

Binding Mode with BCL-XL Target

The binding efficiency of the phytoconstituents from *Cissampelos pareira* against the target BCL-XL is shown in Table 3 and the G-score value ranges from -6.025 to -3.151. All the 13 tested compounds showed good glide score and among that cissamine (**1**), pareitropone (**12**) and pareirubrine B (**11**) showed marked interactions with the G-score value of -6.025, -5.661, -5.587 and glide energy of -45.326, -39.371 and -39.670 respectively. The various hydrogen bonding interactions that bind the constituents of *Cissampelospareira* with the BCL-XL target are ALA (142), ARG (132) ARG (139), ALA (142), GLY (138) and TRP (137).

Binding Mode with NF-κB Protein Target

The binding efficiency of the phytoconstituents from *Cissampelos pareira* against the target NF-κB is shown in Table 4 and the G-score value ranges from -3.785 to -2.010. Out of the 13 tested compounds, six of them showed good glide score and among that l-curine (**9**), cissamperine (**2**) and hayatinine (**6**) showed significant interactions with the G-score value of -3.785, -2.341, -2.350 and glide energy of -36.945, -34.009, -30.893 respectively. The various hydrogen bonding interactions that bind the constituents of *Cissampelospareira* with the NF-κB protein are ASP (206), GLN (50), ARG (51), SER (335), ASP (206) and ASP (235).

Binding Mode with FGF Protein Target

The binding efficiency of the phytoconstituents from *Cissampelos pareira* against the target FGF is shown in Table 5 and the G-score value ranges from -6.682 to -3.310. All the 13 tested compounds showed good glide score and among that pareitropone (**12**), pareirubrine B (**11**) and grandirubrine (**5**) showed marked interactions with the G-score value of -6.682, -6.594, -6.443 and glide energy of -50.164, -51.336, -53.420 respectively. The various hydrogen bonding interactions that bind the constituents of *Cissampelospareira* with the FGF protein are LYS (514), ASP (641), ALA (564), ASP (541), GLU (571), GLN (491), ASN (568) and ASN (659).

Binding Mode with VEGF Protein Target

The binding efficiency against the target VEGF is shown in Table 6. The G-score value ranges from -2.632 to -0.862. Out of 13 tested compounds, eight compounds showed good glide and among that Pareitropone, l-curine and Hayatinine showed significant interaction with target molecule with the G-score value of -2.632, -2.138, -1.785 and Glide energy of -12.755, -23.818, -16.499 respectively. The various hydrogen bonding interactions that bind the constituents of *Cocculushirsutus* with the VEGF protein are TRY (99), GLU (101) and GLN (87).

From the results it is observed that some of the compounds like pareitropone (**12**) and pareirubrine B (**11**) of *Cissampelos pareira* are found to have good glide score and glide energy towards Aurora Kinase, c-Kit, Fibroblast Growth Factor (FGF), Nuclear Factor kappa B (NF-κB), B-cell lymphoma-extra large (Bcl-xL) and Vascular Endothelial Growth Factor (VEGF) as shown in the table.

Aurora kinase which is crucial for cell cycle control is overexpressed in tumour cells [16]. Inhibiting Aurora kinase in such increased levels will control the rapid cell growth. C-kit receptor is present in hematopoietic cells and other tissue cells. C-kit signaling plays a vital role in regulation of the red blood cell production, lymphocyte proliferation etc. Downstream signal transduction regulates cell growth, proliferation and differentiation. Overexpression of BCL-XL relates with resistance to chemotherapy and radiation therapy in multiple cancer [18]. Nuclear factor kappaB (NF-kappaB) is a transcription factor, plays a vital role in carcinogenesis. Therefore inhibition of NF-kappaB activation plays a tumor suppressor role in Liver [19]. FGF target is the Fibroblast Growth Factor which will increase in solid, and metastatic tumors and gives drug resistance. Thus inhibitions of FGF will results in the entry of chemical compounds in to the target cell and thereby cell toxicity and retardation of cell growth [20]. VEGF-receptors are the vascular endothelial growth receptors useful of the vasculogenesis *i.e.* formation of the circulatory system and angiogenesis i.e growth of blood vessels from pre-existing vasculature. Over expression of VEGF will lead to the abnormal growth of tumour cells. Therefore inhibitor of VEGFR will control the growth of new cells binding with the above targets and inhibiting their activity will shows a significant tumour control in liver tissues. Therefore the present study, suggests that these phytoconstituent (**12**) especially the best candidates like pareitriponeand pareirubrine B (**11**) will exhibit its activity by effective binding with the targets as explained by GLIDE software.

4.0 CONCLUSION

The present study suggests that among the various compounds docked, pareitropone (**12**), dicentrine (**4**) and pareirubrine B (**11**) showed the best interaction with aurorakinase. Cissamine (**1**), pareirubrine B (**11**) and grandirubrine (**5**) showed good interaction with C-kit. Cissamine (**1**), pareitropone (**12**) and pareirubrine B (**11**) showed good interaction with BCL-XL. l-curine (**9**), cissamperine (**2**) and hayatinine (**6**) has good interaction with NF-kB. Pareitropone (**12**), pareirubrine B (**11**) and grandirubrine (**5**) showed

good interaction with FGF. Pareitripone (**12**), l-curine (**9**) and hayatinine (**6**) showed good interaction with VEGFR. The binding efficiency of all these compounds with the cancer targets were good. Therefore these best candidates have to be explored further to find a solution for hepato carcinoma.

Table 1 Docking of Reported Phytoconstituents of *C. pareira* with Aurora Kinase

S. No	Compounds	Glide Score	Glide Energy	Interactions	Bond Distance (DH...A) A°
1.	Cissamine	-6.428	-50.359	LYS (143) ALA (213)	1.882 2.400
2.	Cissamperine	-4.038	-45.240	-	-
3.	Cycleanine	-2.634	-33.074	-	-
4.	Dicentrine	-8.242	-56.106	ALA (213), LYS (143) LEU (139)	1.948, 2.237 2.355
5.	Grandirubrine	-6.368	-55.189	LYS (143), LYN (162)	1.811, 2.432
6.	Hayatinine	-5.958	-57.574	LYS (141)	1.914
7.	Insularine	-3.168	-36.381	-	-
8.	Isochondrodendrine	-6.161	-56.708	LEU (139), LYS (141)	1.713, 1.823
9.	l-curine	-5.642	-55.526	ALA (213)	2.023
10.	Pareirubrine A	-7.133	-56.341	LYS (143), LYN (162)	2.380, 2.056
11.	Pareirubrine B	-7.241	-56.325	LYN (162), LYS (143)	2.202, 2.194
12.	Pareitropone	-8.355	-57.650	-	-

Table 2 Docking of Phytoconstituents of *C. pareira* with C-kit

S. No	Compounds	Glide Score	Glide Energy	Interaction	Bond Distance (DH...A) A°
1.	Cissamine	-7.318	-54.064	CYS (674)	1.806
2.	Cissamperine	-	-	-	-
3.	Cycleanine	-3.012	-26.896	-	-
4.	Dicentrine	-4.890	-38.600	-	-
5.	Grandirubrine	-7.238	-56.052	CYS (673)	1.909
6.	Hayatinine	-4.551	-44.657	LYS (593)	1.827
7.	Insularine	-	-	-	-
8.	Isochondrodendrine	-4.368	-41.601	LEU (595)	1.950
9.	l-curine	-5.570	-48.319	ASN (680)	2.454
10.	Pareirubrine A	-6.827	-51.316	CYS (673)	2.057
11.	Pareirubrine B	-7.305	-53.594	CYS (673) TYR (672)	1.928 2.245
12.	Pareitropone	-6.998	-52.404	LYS (593) CYS (673)	2.491 2.043

Table 3 Docking of Phytoconstituents of *C. pareira* with BCL-XL

S. No	Compounds	Glide Score	Glide Energy	Interaction	Bond Distance (DH...A) A°
1.	Cissamine	-6.025	-45.326	ALA (142) ARG (132)	2.192 1.671
2.	Cissamperine	-4.127	-39.124	ARG (139) ARG (139)	1.748 2.074
3.	Cycleanine	-3.151	-29.334	-	-
4.	Dicentrine	-5.043	-36.546	-	-
5.	Grandirubrine	-5.336	-41.810	-	-
6.	Hayatinine	-5.825	-50.222	ALA (142)	2.282
7.	Insularine	-	-	-	-
8.	Isochondrodendrine	-4.067	-40.723	-	-
9.	l-curine	-5.615	-54.363	ARG (132)	1.888
10.	Pareirubrine A	-4.775	-36.138	-	-
11.	Pareirubrine B	-5.587	-39.670	-	-
12.	Pareitropone	-5.661	-39.371	-	-

Table 5 Docking of Phytoconstituents of *C. pareira* with NF-κB

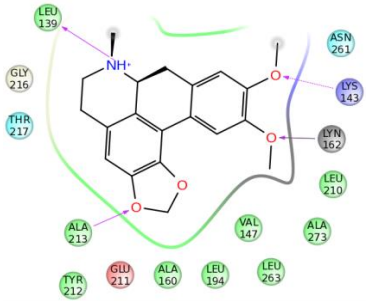
S. No	Compounds	Glide Score	Glide Energy	Interaction	Bond Distance (DH...A) A°
1.	Cissamine	-	-	-	-
2.	Cissamperine	-2.341	-34.009	ASP (206)	2.259
3.	Cycleanine	-2.342	-32.232	-	-
4.	Dicentrine	-	-	-	-
5.	Grandirubrine	-	-	-	-
6.	Hayatinine	-2.350	-30.893	-	-
7.	Insularine	-	-	-	-
8.	Isochondrodendrine	-2.010	-27.117	GLN (50) ARG (51)	2.115 2.187
9.	l-curine	-3.785	-36.945	ARG (51) SER (335) ASP (206)	2.056 2.434 1.988
10.	Pareirubrine A	-	-	-	-
11.	Pareirubrine B	-	-	-	-
12.	Pareitropone	-	-	-	-

Table 6 Docking of Phyto-constituents of *C. pareira* with FGF

S. No	Compounds	Glide Score	Glide Energy	Interactions	Bond Distance (DH...A) A°
1.	Cissamine	-6.330	-52.304	LYS (514) ASP (641) ALA (564)	2.208 2.009 1.684
2.	Cissamperine	-4.512	-41.798	ASP (641)	2.057
3.	Cycleanine	-3.488	-46.964	-	-
4.	Dicentrine	-6.397	-48.112	LYS (514) ALA (564)	2.176 1.929
5.	Grandirubrine	-6.443	-53.420	LYS (514)	1.955
6.	Hayatinine	-5.948	-59.796	ASP (641)	1.798
7.	Insularine	-3.310	-48.854	-	-
8.	Isochondrodendrine	-5.615	-66.647	ASP (541) GLU (571) GLN (491)	1.556 2.308 2.476
9.	l-curine	-5.527	-61.197	-	-
10.	Pareirubrine A	-	-	-	-
11.	Pareirubrine B	-6.594	-51.336	LYS (514)	1.758
12.	Pareitropone	-6.682	-50.164	LYS (514) ALA (564)	1.748 2.874

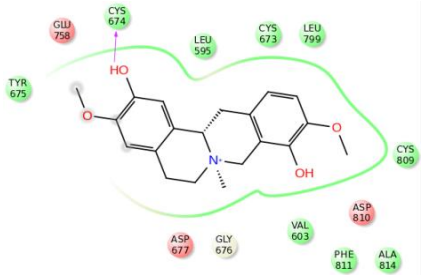
Table 7 Docking of Phytoconstituents of *C. pareira* with VEGF

S. No	Compounds	Glide Score	Glide Energy	Interactions	Bond Distance (DH...A) A°
1.	Cissamine	-2.500	-17.280	TRY (99)	2.196
2.	Cissamperine	-1.088	-15.171	-	-
3.	Cycleanine	-1.571	-17.648	GLU (101)	1.855
4.	Dicentrine	-	-	-	-
5.	Grandirubrine	-	-	-	-
6.	Hayatinine	-1.785	-16.499	GLU (101)	1.672
7.	Insularine	-	-	-	-
8.	Isochondrodendrine	-0.862	-10.363	GLN (87)	2.443
9.	l-curine	-2.138	-23.818	TRY (99)	1.918
10.	Pareirubrine A	-	-	-	-
11.	Pareirubrine B	-	-	-	-
12.	Pareitropone	-2.632	-12.755	GLN (87) GLN (87)	2.022 2.414



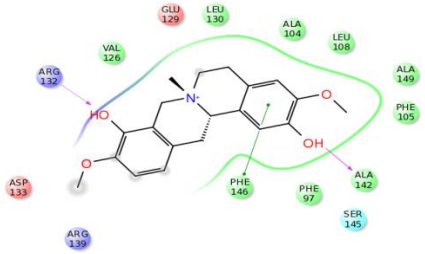
Glide score : -6.397
Glide energy : -48112
Interactions : ALA (213), LYS (143), LEU (139)

Figure 2 Docking of Aurora Kinase with Dicentrine **(4)**



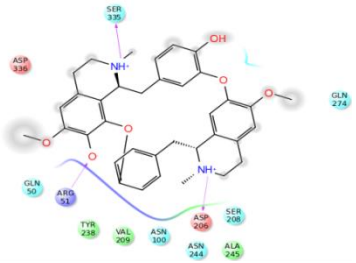
Glide score : -7.318
Glide energy : -54.064
Interactions : CYS(674)

Figure 3 Docking of C-Kit with Cissamine **(1)**



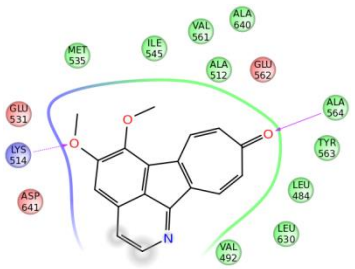
Glide score : -6.025
Glide energy : -45.326
Interactions : ALA (143), ARG (132)

Figure 4 Docking of BCL-XL with Cissamine **(1)**



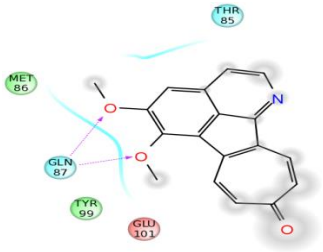
Glide score : -3.785
Glide energy : -36.945
Interactions : ARG(51), SER(335), ASP (206)

Figure 5 Docking of NF-κB with I-curine **(9)**



Glide score : -6.682
Glide energy : -50.164
Interactions : LYS (514), ALA (564)

Figure 6 Docking of FGF with Pareitropone **(12)**



Glide score : -2.632
Glide energy : -12.755
Interactions : GLN (87), GLN (87)

Figure 7 Docking of VEGF with Pareitropone **(12)**

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