

Title: In Silico Study of Silybum Marianum Targeting PARP protein (4UND protein)**Authors: Hrishikesh D. GOSAVI¹; Dr. Pravin BADHE²**¹*Student, Department of Pharmacy, Navsahyadri Institute of Pharmacy, Pune, INDIA.*²*Associate Professor, Pharmaceutics Department, Navsahyadri Institute, of Pharmacy, Pune, INDIA.**Affiliations: Mr. Hrishikesh Gosavi; Dr. Pravin Badhe.**Corresponding Authors E-Mail- gosavihrishikesh@gmail.com***Abstract-**

Silybum Marianum, is a plant belonging to the family Asteraceae. For many centuries it has been used a natural remedy for many diseases like Liver and Biliary tract diseases. It is effective as an anti-oxidant and is used in a variety of diseases. This study was conducted to check the effects of Silybum Marianum on PARP protein (4UND protein).

The Molecular Docking techniques was chosen to check the effects of different chemical constituents of Silybum marianum on DNA damaging protein. For this purpose, different PARP inhibitor drugs were taken as standard. The Molecular Docking of the chemical constituents of Silybum marianum was performed using 4UND protein with the help of PyRx software along with BIOVIA Drug Discovery studio software. The result of molecular docking showed that some of the chemical constituent have higher binding affinity than standard PARP inhibitor drugs.

Keywords-

Silybum Marianum, Parp protein, 4UND protein, Molecular Docking, PyRx, BIOVIA Drug Discovery.

Introduction-

Milk thistle which is also known as *Silybum Marianum*, belongs to the family of *Asteraceae* and is an annual or biennial herbaceous plant local of the Mediterranean basin, it is these days widely spread in Central Europe, America and South-Australia [1][2]. It can also be found in India, China, and Africa [3]. There are two species of *Genus Silybum(L). Gaertn* - *S.Marianum* with variegated leaves and *S. Eburneum* Coss & Dureu with completely green leaves [4]. It grows to a peak of 60–150 cm

and it has white-veined leaves with toothed spiny edges and purple flowers on the pinnacle of the stem and the ramifications ripe into 6–7 mm long brownish fruits (achenes) with a white silky pappus at their apex and has big prickly edged leaves included with undulating white patches and stems containing a milky juice [5][6][7].



[4]

Fig. Silybum Marianum

Milk Thistle's active component is a lipophilic extract obtained from the seeds of the plant, and it contains three isomer flavonolignans (silybin, silydianin, and silychristin), together known as silymarin [8][9].

Because of the existence of phytoconstituents such as antioxidants and complete phenolics, milk thistle is used as a medicine for the treatment of a variety of diseases [9]. For years, *Silybum Marianum* has been used as a natural treatment for liver and biliary tract diseases [10]. A study that was done was to see how effective *Silybum Marianum* is at scavenging free radicals in vitro and how powerful it is as an antioxidant [11]. Milk thistle extracts are currently being researched for chemoprevention, recovery, and amelioration of chemotherapy side effects in cancer experimental therapeutics, but however, nomenclature precision has fallen behind [12]. Preclinical evidence for silymarin's hepatoprotective and anti-carcinogenic effects, includes cancer cell growth inhibition in humans, prostate, skin, breast and cervical cells [13]. Extracts from milk thistle seeds have shown efficacy in halting the progression of human prostate carcinoma in a variety of in vitro and in vivo preclinical

models over the last few years [14]. It has also been proposed that Milk Thistle can be used in diabetes to diminish insulin resistance [15].

NAD⁺ protein (ADP-ribosyl) transferase (polymerizing) (PARP) is a widely distributed chromatin-associated protein that is highly conserved and found in all eukaryotes except yeast [16]. PARP attaches to DNA strand, breaks and catalyses the transfer of ADP ribose from its substrate NAD⁺ to a number of nuclear acceptors, mostly to itself, in response to DNA damage induced by external genotoxic chemicals and endogenous cellular processes [17]. PARP-1 uses its two zinc fingers to detect DNA nicks, which it catalytically activates by binding to the break and helps to repair. The only other PARP enzyme activated by and assisting in the repair of DNA breaks is PARP-2, which has the closest structural resemblance to PARP-1 [18][19]. Of all the proven use of PARP inhibitors the most promising ones are ischemia and cancer [20]. So, we have decided to do the research on cancer.

While doing this research we have identified the research gap that no one else has used 4UND protein before for Molecular Docking of chemical constituents of Silybum Marianum on anti-cancer activity so, we have used 4UND protein.

Based on the above information, we intend to carry out in silico study of chemical constituents of Silybum Marianum with PARP protein. We are doing this study so that we can check the binding affinity of chemical constituents of Silybum Marianum and compare it with the binding affinity of present PARP inhibitor drugs i.e., Talazoparib, Rucaparib, Olaparib, Niraparib and if the results acquired are good, we can create new drugs which can show better anti-cancer activity than present drugs which are available.

The purpose of this study was to know about the docking affinity of the chemical constituents of Silybum Marianum. The Molecular Docking was done by using the PyRx software and BIOVIA Drug Discovery. The necessary proteins and ligands were downloaded from protein bank and PubChem respectively.

Botanics-

Botanical Name- Silybum Marianum

Kingdom- Plantae

Division- Magnoliophyta

Class- Magnoliopsida

Subclass- Asteridae

Order- Asterales

Family- Asteraceae

Genus- Silybum

Species- Silybum Marianum [21]

Chemical Constituents of *Silybum Marianum*-

Oil content 26.05%, moisture 4.48%, ash 1.93%, crude fibre 5.48%, carbs 87.2%, and total proteins 23% make up Silybum Marianum seeds. Apigenin, silybinol, proteins, betaine, fixed oil, and free fatty acids are active elements found in the seeds of the milk thistle. It also contains sugars such as arabinose, rhamnose, xylose, glucose [4][9].

The main bioactive component of medicinal plant (milk thistle) is silymarin (4% to 6%). Silymarin is the mixture of different flavonolignans such as, silybinin (60%) A and B (SBN A&B), iso-silybinin A and B (ISBN A&B), silychristin (SCN), and silydianin (SDN), it is a well-known Chinese herb. Silymarin is also present in the seeds, fruits as well as in the leaves of the Milk Thistle but the seed part has the maximum concentration of silymarin. Besides this Milk Thistle also includes other flavonoids such as Rutin, quercetin, dihydrokaempferol, kaempferol, apigenin, naringin, eriodictiol, chrysoeriol [4][9][22].

Other constituents are fixed oil (16% to 18%), betaine, trimethyl-glycine and amines, 20% - 30% fixed oil, of which approximately 60% is linoleic acid, glutamic acid, approximately 30% is oleic acid, and approximately 9% palmitic acid, 25-30% protein, 0.038% tocopherol, 0.63% sterols- including cholesterol, campesterol, stigmasterol, and sitosterol, some mucilage, 2,2-diphenyl-1- picrylhydrazyl, [22]. The fatty acids that are present are saturated fatty acids- Lauric (C12:0), Myristic (C 14:0), Palmitic (C 16:0), Stearic (C 18:0), Arachidic (C 20:0), Behenic (C 22:0), Lignoceric (C 24:0), Palmitoleic (C 16:1), Heptadecenoic (C 17:1), Oleic (C 18:1), Vaccenic (C 18:1), Eicosenoic (C 20:1), Erucic (C 22:1).

Polyunsaturated fatty acids are- Linoleic, Linolenic. The ash content is 9.6% in stalks, 10.5% in leaves and 9.7% in heads [23].

The essential amino acids present in Silybum Marianum are- Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Valine, Histidine, and Non-Essential amino acids present are- Alanine, Aspartic acid, Glutamic acid, Glycine, Proline, Serine, Arginine, Cysteine, Tyrosine [23].

The mineral content of Silybum Marianum is-

- Na – 81.0
- K – 718.0
- Ca – 132.0
- Mg – 17.3
- Cu – 0.08
- Fe – 0.50
- Mn – 0.10
- Zn – 0.26 [23].

Molecular Docking-

The field of molecular docking has grown in popularity over the last three decades as a result of the structural molecular biology and structure-based drug discovery requirements [24]. Molecular docking is a type of computational modelling that helps predict the preferred binding orientation of one molecule (e.g., ligand) to another (e.g., receptor) when they interact to form a stable complex [25].

Materials-

Hardware & Software- HP 15-da0300TU with Intel core CPU of 2.57 Ghz, 8 gb ram, and intel integrated graphics card of 4gb, and 64-bit windows operating system.

PyRx a software [26] written in python language, which today can be used on any PC (personal computer), is used along with BIOVIA Drug Discovery software [27] for molecular docking.

The files used for molecular docking were ligands which were downloaded from PubChem [28] in “sdf” format and Proteins which were downloaded from Protein bank in “pdb” format [29].

Methods-

The protein files that were downloaded were first converted to “pdbqt” format from “pdb” format. The conversion of file can be done in the PyRx software which includes Auto Dock Vina.

- 1- Start by double clicking on PyRx icon on your desktop.
- 2- Select file option on the right-hand side corner of your screen, and then choose the “Load Molecule” option and select your protein.
- 3- Then right click on the selected protein and choose the Auto Dock option and select the “Make Macromolecule” option.
- 4- Then select the “Open Babel” option on the lower side of your screen. Then click on the right side first icon of insert new item, then select the ligands one by one.
- 5- Then right click on the selected ligand and choose the Minimize All option.
- 6- After that right click again and select the “convert all to AutoDock Ligand(pdbqt) option.
- 7- Then select the vina wizard and click on start.
- 8- Select the ligands using cntrl button on your keyboard and and using cursor.
- 9- Then on the screen the structure will appear make sure the whole structure is in the box.
- 10-Then click on Forward button and the docking will start.
- 11-After completion of the docking process click on “save as comma-separated vale” on the right side of your screen, and the binding affinity results will be saved in excel sheet.

Results-

The results that are given below shows the binding affinity between **Standard PARP inhibitor drugs** i.e., Talazoparib, Rucaparib, Olaparib, and Niraparib with **PARP protein (4UND protein)** and Chemical constituents of **Silybum Marianum** i.e., Silychristin, Silydianin, and Naringin with **PARP protein (4UND protein)**.

Table1 shows the binding affinity of standard PARP inhibitor drugs with 4UND PARP protein and Table 2 shows the binding affinity of chemical constituents of Silybum Marianum with 4UND PARP protein.

A) Standard**Table-1.**

| Sr.No | Complex (Protein + Ligand) | Ligands | Binding affinity (kcal/mol) |
|-------|---------------------------------|-------------|-----------------------------|
| 1 | 4und_(1)_135565082_uff_E=560.92 | Talazoparib | -8.9 |
| 2 | 4und_(1)_9931954_uff_E=653.31 | Rucaparib | -7.8 |
| 3 | 4und_(1)_23725625_uff_E=1707.13 | Olaparib | -9.8 |
| 4 | 4und_(1)_24958200_uff_E=461.77 | Niraparib | -8.2 |

B) Chemical Constituents

| Sr.No | Complex (protein + Ligand) | Ligands | Binding Affinity (kcal/mol) |
|-------|--------------------------------|--------------|-----------------------------|
| 1 | 4und_(1)_441764_uff_E=498.05 | Silychristin | -10.1 |
| 2 | 4und_(1)_11982272_uff_E=629.25 | Silydianin | -11.2 |
| 3 | 4und_(1)_442428_uff_E=615.92 | Naringin | -11.2 |

Table- 2.

The binding affinity of the other chemical constituents of Silybum Marianum which are also found to be good but not better than the standard PARP inhibitor drugs has been shown in Table 3 in appendix section as well as the binding affinity of the chemical constituents of Silybum Marianum whose binding affinity was found to be less than -6 is shown in Table 4 in appendix.

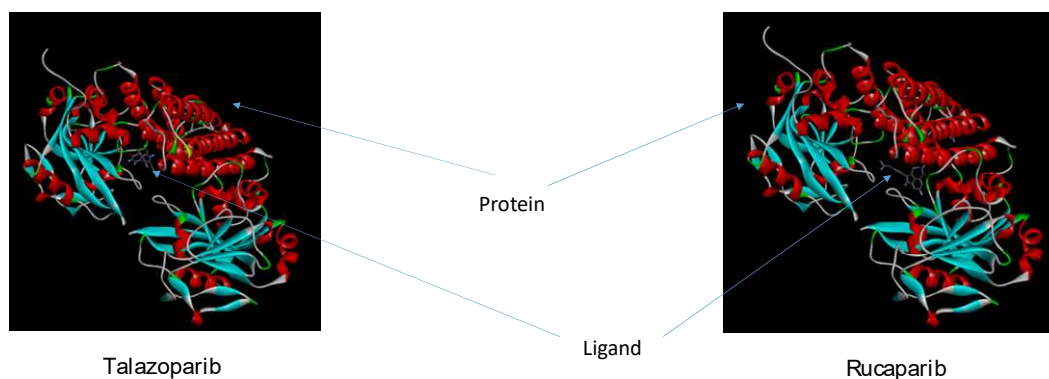


Fig. Binding of Standard PARP inhibitor drugs with 4UND protein

Figure- 1.

Figure 1 shows the binding between Standard PARP inhibitors i.e., Talazoparib and Rucaparib with 4UND protein (Table- 1). The binding affinity of Talazoparib with 4UND protein was found to be -8.9 kcal/mol and the binding affinity of Rucaparib with 4UND protein was found to be -7.8 kcal/mol. The figure shows the protein and the ligand marked with arrows. The Red, blue, yellow and green colour section of the figure shows the protein i.e., 4UND PARP protein and the violet colour section shows the ligands i.e., Talazoparib and Rucaparib.

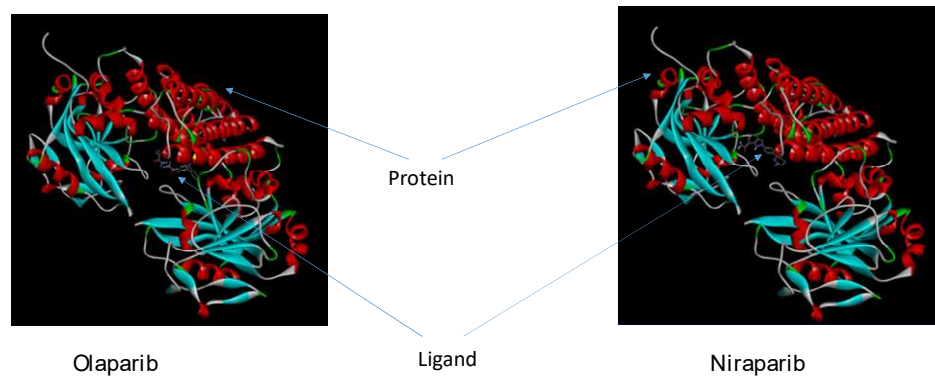


Fig. Binding of Standard PARP inhibitor drugs with 4UND protein

Figure- 2.

Figure 2 shows the binding between Standard PARP inhibitors i.e., Olaparib and Niraparib with 4UND protein (Table- 1). The binding affinity of Olaparib with 4UND protein was found to be -9.8 kcal/mol and the binding affinity of Niraparib with 4UND protein was found to be -8.2 kcal/mol. The figure shows the protein and the ligand marked with arrows. The Red, blue, yellow and green colour section of the figure shows the protein i.e., 4UND PARP protein and the violet colour section shows the ligands i.e., Olaparib and Niraparib.

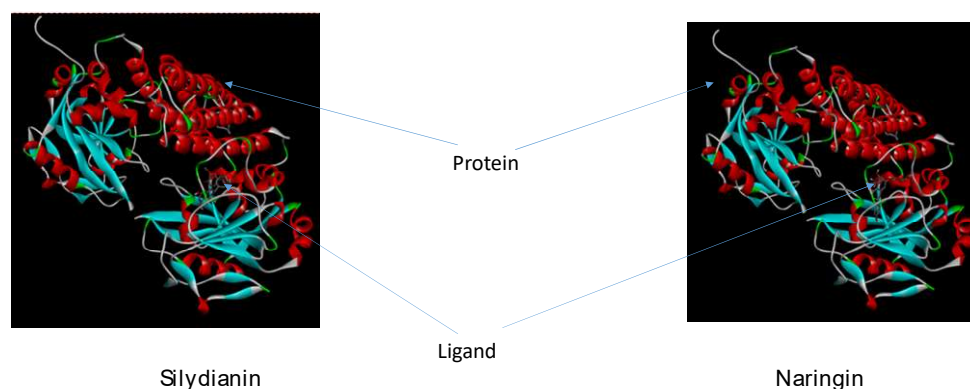


Fig. Binding of Chemical Constituents of Silybum Marianum with 4UND protein (This figure shows chemical constituents of Silybum Marianum with best binding affinity with 4UND protein)

Figure- 3.

Figure 3 shows the binding between Chemical constituents of Silybum Marianum i.e., Silydianin and Naringin with 4UND protein (Table- 2). The binding affinity of Silydianin with 4UND protein was found to be -11.2 kcal/mol and the binding affinity of Naringin with 4UND protein was found to be -11.2 kcal/mol. The binding affinity of Silydianin and Naringin with 4UND protein was found to be better than the binding affinity of standard PARP inhibitor drugs with 4UND protein. The figure shows the protein and the ligand marked with arrows. The Red, blue, yellow and green colour section of the figure shows the protein i.e., 4UND PARP protein and the violet colour section shows the ligands i.e., Silydianin and Naringin.



Fig. Binding of Chemical Constituents of Silybum Marianum with 4UND protein (This figure shows chemical constituents of Silybum Marianum with best binding affinity with 4UND protein)

Figure- 4.

Figure 4 shows the binding between the Chemical Constituent of Silybum Marianum i.e., Silychristin with 4UND protein (Table-2). The binding affinity of Silychristin with 4UND protein was found to be - 10.1 kcal/mol. The binding affinity of Silychristin with 4UND protein was found to be better than the binding affinity of standard PARP inhibitor drugs with 4UND protein. The figure shows the protein and the ligand marked with arrows. The Red, blue, yellow and green colour section of the figure shows the protein i.e., 4UND PARP protein and the violet colour section shows the ligands i.e., Silychristin.

The remaining images of binding of chemical constituents of Silybum Marianum with 4UND protein are added in the Appendix section.

From the above results it can be said that, Silydianin, Naringin, and Silychristin which are the chemical constituents of Silybum Marianum shows better binding affinity than Talazoparib, Rucaparib, Olaparib, and Niraparib. Talazoparib, Rucaparib, Olaparib, and Niraparib all are Standard PARP inhibitor drugs.

Silymarin, Silybinin, Apigenin, Quercetin and Campesterol all are the chemical constituents of Silybum Marianum and shows better binding affinity than Talazoparib. Silymarin, Silybinin, Apigenin, Quercetin,

Campesterol, and Sitosterol, shows better binding affinity than Rucaparib. Silymarin, Silybinin, Apigenin, Quercetin, Campesterol and Sitosterol, shows better binding affinity than Niraparib.

Discussion-

For centuries, Silybum Marianum has been used in the treatment of various diseases mainly liver and biliary tract related diseases. It has been used as a natural remedy for all these centuries. In this study carried out by using Molecular Docking the binding affinity of chemical constituents of Silybum Marianum with 4UND PARP protein was carried out. The binding affinity of Silydianin and Naringin which are the chemical constituents of Silybum Marianum showed good interaction with 4UND protein.

The binding affinity was found out to be as followed:

The binding affinity of Standard PARP inhibitor drugs was found to be-

- 1- Talazoparib: -8.9 kcal/mol.
- 2- Rucaparib: -7.8 kcal/mol.
- 3- Olaparib: -9.8 kcal/mol.
- 4- Niraparib: -8.2 kcal/mol.

As we can see the best binding affinity among Standard PARP inhibitor drugs was found to be of OLAPARIB which is -9.8 kcal/mol.

The binding affinity of chemical constituents of Silybum Marianum was found to be as followed:

- 1- Silymarin: -9.4 kcal/mol.
- 2- Silybinin: -9.3 kcal/mol.
- 3- Silychristin: -10.1 kcal/mol.
- 4- Silydianin: -11.2 kcal/mol.
- 5- Apigenin: -9.2 kcal/mol.
- 6- Quercetin: -9.3 kcal/mol.
- 7- Dihydrokaempferol: -7.2 kcal/mol.
- 8- Kaempferol: -7.4 kcal/mol.
- 9- Naringin: -11.2 kcal/mol.
- 10-Eriodictol: -7.8 kcal/mol.

11-Chrysoeriol: -7.6 kcal/mol.

12-Campesterol: -9.1 kcal/mol.

13-Sitosterol: -8.8 kcal/mol.

14- Rutin: -8.9 kcal/mol.

As we can see the binding affinity of **Silydianin** which is found to be -11.2 kcal/mol and the binding affinity of **Naringin** which was also found to be -11.2 kcal/mol which is the best amongst all the chemical constituents of Silybum Marianum. From this we can say that the binding affinity of Silydianin and Naringin was found to be better than the binding affinity of Olaparib. And also, the binding affinity of **Silychristin** which is -10.1 kcal/mol which is also better than Olaparib, Niraparib, Rucaparib and Talazoparib.

The previous proven activities of Silydianin, Naringin and Silychristin are- **Silydianin** which is an active chemical constituent of Silybum Marianum is proven to have inhibitory effect [30], antioxidant and cytotoxic activities in Caco-2 cells [31], used in prostate cancer [32], PTP1B inhibitor [33]. **Naringin** which is also a chemical constituent of Silybum Marianum shows antioxidant, anti-inflammatory, anti-apoptotic, anti-ulcer, anti-osteoporotic and anti-carcinogenic activity [34], Anti-bacterial [35], it regulates p21 and survivin in triple negative breast cancer cells [36]. **Silychristin** which is also a chemical constituent of Silybum Marianum shows anti-oxidant, anti-inflammatory and Multidrug Resistance Modulation Activity [37], used in cancer prevention [38]. So, from these proven activities we can say that Silydianin, Naringin and Silychristin are previously being used for their anti-cancer activity and from the results of molecular docking we can also say that they can be used as PARP inhibitors in the future.

The binding affinity of Silymarin -9.4 kcal/mol, Silybinin -9.3 kcal/mol, Apigenin -9.2 kcal/mol, Quercetin -9.3 kcal/mol and Campesterol -9.1 kcal/mol was found to be better than Talazoparib -8.9 kcal/mol. The binding affinity of Rutin -8.9 kcal/mol was found to be equal to Talazoparib.

The binding affinity of Sitosterol -8.8 kcal/mol was to be better than Rucaparib which is -7.8 kcal/mol. The binding affinity of Eriodicytol -7.8 kcal/mol was found to be equal to Rucaparib. The binding affinity of Silymarin -9.4 kcal/mol, Silybinin -9.3 kcal/mol, Apigenin -9.2 kcal/mol, Quercetin -9.3 kcal/mol, Campesterol -9.1 kcal/mol, Sitosterol -8.8 kcal/mol and Rutin -8.9 kcal/mol was found to be better than Niraparib -8.2 kcal/mol.

PARP inhibitors are a cancer therapy targeting poly (ADP-ribose) polymerase were the first drugs to get approval to exploit synthetic lethality. The standard PARP inhibitor Drugs used were Talazoparib, Rucaparib, Olaparib, and Niraparib [39].

Olaparib was the first PARP inhibitor drug to get approved by European Medicines Agency in 2014, for mutant ovarian cancer [40] it also shows effect in late chemotherapy-refractory BRCA-deficient breast cancer and also olaparib is well tolerated and very active [41]. Rucaparib a PARP inhibitor drug is known to be tolerable and shows activity in patients with platinum-sensitive germline BRCA ½ mutated-HGOC [42]. Talazoparib which is a PARP inhibitor drug inhibits PARP catalytic activity, trapping PARP1 on damaged DNA and causing cell death in *BRCA* ½ -mutated cells [43]. Niraparib a potent PARP ½ inhibitor drug with good cell activity shows promising activity in cancer patients [44].

So, on the basis of the result obtained we can expect that Silydianin and Naringin also possess the inhibition activity against Oxidative DNA damage, because Silybum marianum extract has the ability to show protection against photo-carcinogenesis [45] and also on the basis of the results of molecular docking we can expect that in the future Silybum Marianum can be used to create PARP inhibitor drugs.

Conclusion-

The analysis of the results of Molecular Docking of chemical constituents of Silybum Marianum with 4UND DNA damaging protein helped to examine and evaluate the binding affinity of chemical constituents of Silybum Marianum with 4UND DNA damaging protein. The results obtained showed that Silydianin and Naringin the chemical constituents of Silybum Marianum showed the binding affinity of -11.2 kcal/mol respectively, which is better than the best binding affinity amongst the Standard PARP inhibitor drugs which was shown by Olaparib which is -9.8 kcal/mol.

From the above result we can conclude that Silydianin and Naringin, the chemical constituents of Silybum Marianum showed better binding affinity than Talazoparib, Rucaparib, Olaparib, and Niraparib which are the standard PARP inhibitor drugs. Also, Silychristin which is a chemical constituent of Silybum Marianum shows the binding affinity of -10.1 kcal/mol, which is also better than Talazoparib, Rucaparib, Olaparib, and Niraparib. So, we can say that in the future we can expect to use Silybum Marianum to create PARP inhibitor drugs.

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Appendix-

| Sr.No | Complex (protein + ligand) | Ligands | Binding Affinity (kcal/mol) |
|-------|-------------------------------|-------------------|-----------------------------|
| 1 | 4und_(1)_5213_uff_E=444.88 | Silymarin | -9.4 |
| 2 | 4und_(1)_31553_uff_E=443.51 | Silybinin | -9.3 |
| 3 | 4und_(1)_5280443_uff_E=233.26 | Apigenin | -9.2 |
| 4 | 4und_(1)_5280343_uff_E=380.43 | Quercetin | -9.3 |
| 5 | 4und_(1)_122850_uff_E=233.92 | Dihydrokaempferol | -7.2 |

| | | | |
|----|-------------------------------|-------------|------|
| 6 | 4und_(1)_5280863_uff_E=362.50 | Kaempferol | -7.4 |
| 7 | 4und_(1)_373261_uff_E=224.97 | Eriodictol | -7.8 |
| 8 | 4und_(1)_5280666_uff_E=324.01 | Chrysoeriol | -7.6 |
| 9 | 4und_(1)_173183_uff_E=573.30 | Campesterol | -9.1 |
| 10 | 4und_(1)_222284_uff_E=590.88 | Sitosterol | -8.8 |
| 11 | 4und_(1)_5280805_uff_E=751.59 | Rutin | -8.9 |

Table-3.

| Sr.No | Complex (protein + ligand) | Ligands | Binding Affinity (kcal/mol) |
|-------|-------------------------------|-------------------|-----------------------------|
| 1 | 4und_(1)_247_uff_E=88.22 | betaine | -3.8 |
| 2 | 4und_(1)_248_uff_E=88.90 | Trimethyl Glycine | -3.8 |
| 3 | 4und_(1)_5280450_uff_E=147.99 | Linoleic acid | -5.9 |
| 4 | 4und_(1)_445639_uff_E=80.35 | Oleic acid | -5.8 |
| 5 | 4und_(1)_11005_uff_E=51.08 | myristic | -4.7 |
| 6 | 4und_(1)_135191_uff_E=154.55 | xylose | -5.4 |
| 7 | 4und_(1)_6137_uff_E=94.18 | methionine | -4.1 |

Table- 4.**Silymarin****Silybinin****Fig. Binding of Chemical Constituents of Silybum Marianum with 4UND protein**



Dihydrokaempferol

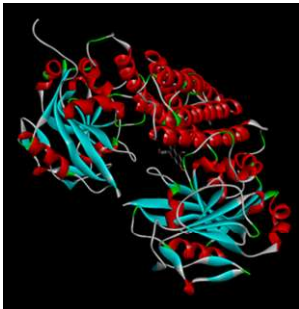


Kaempferol

Fig. Binding of Chemical Constituents of Slybum Marianum with 4UND protein



Dihydrokaempferol



Kaempferol

Fig. Binding of Chemical Constituents of Slybum Marianum with 4UND protein

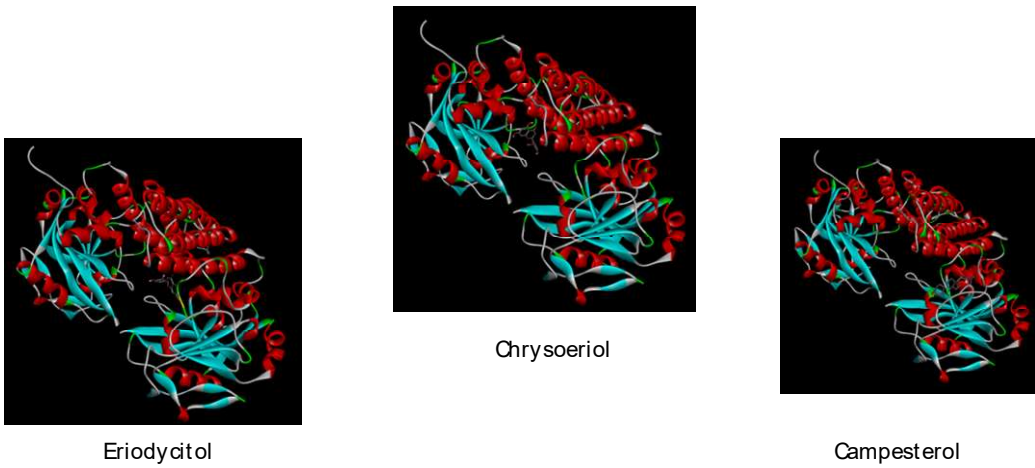


Fig. Binding of Chemical Constituents of Slybum Marianum with 4UND protein

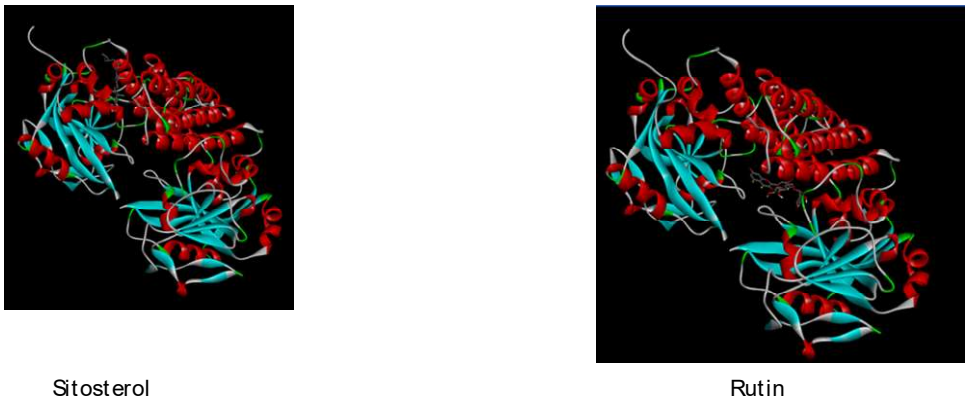


Fig. Binding of Chemical Constituents of Slybum Marianum with 4UND protein