e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

International Journal of Pharmaceutical Research & Analysis
www.ijpra.com

Research Article

IN-SILICO EVALUATION OF SILYBUM MARIANUM PHYTOCONSTITUENTS FOR HEPATOPROTECTIVE ACTIVITY USING MOLECULAR DOCKING

Dr. Saravanan C[1], Janani S[2*]

[1]Professor Cum HOD Department of Pharmaceutical Analysis, Aadhibhagawan College of Pharmacy, Rantham Village, Thiruvannamalai District-604407, Tamilnadu, India.
[2]B. Pharm Final Year, Aadhibhagawan College of Pharmacy, Rantham Village, Thiruvannamalai District-604407, Tamilnadu, India.

ABSTRACT
Silybum marianum (Milk Thistle) is a medicinal plant rich in silymarin, flavonolignans, and flavonoids, which possess significant pharmacological activities. The present study evaluated the molecular docking potential of ten phytoconstituents (Silybin, Isosilybin A, Isosilybin B, Silychristin, Silydianin, Isosilychristin, Taxifolin, Quercetin, Kaempferol, and Apigenin) against target proteins associated with hepatoprotective, cardioprotective, antioxidant, anti-inflammatory, nephroprotective, anticancer, and neuroprotective activities. Molecular docking was performed using AutoDock 4.2, while Discovery Studio Visualizer was used for interaction analysis. ADME and toxicity studies were carried out to evaluate drug-likeness and safety. The docking results revealed that Apigenin, Silydianin, and Silychristin exhibited the best binding affinities with several target proteins, indicating promising therapeutic potential. Overall, the study suggests that Milk Thistle phytoconstituents could serve as potential lead molecules for future drug development, although further in vitro, in vivo, and clinical studies are required for validation.

Keywords: *Silybum marianum, Silymarin, Phytoconstituents, Molecular Docking, ADME Analysis, Hepatoprotective Activity, Antioxidant Activity, Anti-inflammatory Activity, Neuroprotective Activity*.

INTRODUCTION

Medicinal plants have been an important source of therapeutic agents since ancient times and continue to contribute significantly to modern drug discovery. Their therapeutic potential is mainly attributed to a wide variety of naturally occurring bioactive compounds, including flavonoids, phenolic compounds, terpenoids, alkaloids, glycosides, steroids, and lignans. The increasing interest in plant-based medicines has encouraged extensive scientific investigations to identify their phytochemical constituents, pharmacological activities, mechanisms of action, and potential applications in the prevention and treatment of various diseases.Silybum marianum (L.) Gaertn., commonly known as Milk Thistle, is a well-known medicinal plant belonging to the family Asteraceae. It is an annual or biennial herb characterized by an erect, branched stem, large spiny leaves with distinctive white or pale veins, and prominent purple flower heads. The fruits are small achenes with a characteristic pappus. The mature fruits, commonly referred to as seeds, are considered the principal medicinally important part of the plant because they contain a high concentration of biologically active flavonolignans.

Milk Thistle is native to the Mediterranean region and has been widely distributed and cultivated in several parts of Europe, Asia, Africa, Australia, and other regions. The plant commonly grows in dry and sunny environments, grasslands, roadsides, disturbed areas, and

Corresponding Author
Janani S

Email: jananikani4@gmail.com

open fields. Its ability to tolerate different environmental conditions has contributed to its wide geographical distribution. The plant has a long history of medicinal use, particularly in traditional European herbal medicine. The medicinal importance of *Silybum marianum* is primarily associated with its seeds and the flavonolignan complex known as silymarin. Silymarin is not a single compound but a mixture of several closely related flavonolignans, with silybin generally considered its major constituent. Other important components include isosilybin, silychristin, silydianin, and related compounds. The plant also contains flavonoids and phenolic constituents such as taxifolin, quercetin, kaempferol, luteolin, and apigenin. The presence of these diverse phytoconstituents contributes to the broad pharmacological potential of Milk Thistle.

Silymarin has received considerable scientific attention because of its antioxidant, anti-inflammatory, hepatoprotective, cytoprotective, and free-radical-scavenging properties. It has been extensively investigated for its protective effects against different forms of liver injury. The antioxidant activity of Milk Thistle is associated with its ability to scavenge reactive oxygen species and reduce oxidative damage. Its constituents may also influence cellular antioxidant defense mechanisms and help maintain the structural and functional integrity of cells. The hepatoprotective activity of *Silybum marianum* is one of its most extensively studied pharmacological properties. Traditionally, Milk Thistle has been used for various liver and biliary disorders. Experimental investigations have suggested that silymarin may protect hepatic cells against oxidative stress and toxic insults, while also supporting cellular regeneration and maintaining normal liver function. Because of these properties, Milk Thistle has become one of the most widely investigated herbal plants in the field of hepatoprotective drug research.

In addition to hepatoprotective activity, *Silybum marianum* has demonstrated several other pharmacological properties. Studies have reported antioxidant, anti-inflammatory, antidiabetic, cardioprotective, antimicrobial, immunomodulatory, and neuroprotective activities associated with its extracts and individual phytoconstituents. The flavonolignans and flavonoids present in the plant may act through multiple biological pathways, including regulation of oxidative stress, inflammatory mediators, cellular signaling, and apoptosis.The scientific evaluation of medicinal plants requires accurate botanical identification, extraction, phytochemical screening, characterization, standardization, and quality control. Various analytical techniques are used to identify and quantify the active constituents of *Silybum marianum*. Spectroscopic and chromatographic methods such as UV–Visible spectroscopy, Fourier Transform Infrared spectroscopy (FT-IR), High-Performance Liquid Chromatography (HPLC), Gas Chromatography–Mass Spectrometry (GC–MS), Liquid Chromatography–Mass Spectrometry (LC–MS), and Nuclear Magnetic Resonance (NMR) spectroscopy provide valuable information regarding the chemical composition and quality of plant materials and extracts.

Although conventional phytochemical and pharmacological investigations provide important information regarding the biological properties of Milk Thistle, understanding the precise molecular interactions of individual phytoconstituents remains important. In silico approaches such as molecular docking, molecular dynamics simulation, and computational pharmacokinetic prediction have therefore become useful tools in natural-product research. Molecular docking can predict the interaction of plant-derived compounds with specific biological targets and provide information regarding binding affinity, binding orientation, interacting amino acid residues, hydrogen bonds, hydrophobic interactions, and other molecular interactions.

The diverse phytochemical profile of *Silybum marianum*, particularly its rich content of flavonolignans and flavonoids, makes it an important candidate for systematic pharmacological and computational investigation. Major constituents such as silybin, isosilybin, silychristin, silydianin, taxifolin, quercetin, kaempferol, luteolin, and apigenin may interact with different molecular targets involved in oxidative stress, inflammation, apoptosis, metabolic dysfunction, and cellular injury. Investigation of these interactions may provide a better understanding of the molecular basis of the therapeutic properties of Milk Thistle.

Therefore, *Silybum marianum* represents a promising medicinal plant for the discovery and evaluation of bioactive natural compounds. Integration of pharmacognostic evaluation, phytochemical characterization, pharmacological studies, and computational approaches can provide comprehensive evidence regarding its therapeutic potential. Such investigations may facilitate the identification of promising phytoconstituents from Milk Thistle and provide a scientific basis for their further development as potential therapeutic agents.

Plant Profile:

Figure 1: Milk Thistle (Silybum marianum).

Morphological Characters

1. Scientific Name: Silybum marianum (L.) Gaertn.
2. Common Name: Milk Thistle
3. Family: Asteraceae

Taxonomical Classification

1. Kingdom: Plantae
2. Division: Magnoliophyta
3. Class: Magnoliopsida
4. Order: Asterales
5. Family: Asteraceae
6. Genus: Silybum
7. Species: Silybum marianum

DESCRIPTION AND DISTRIBUTION:

Milk thistle is an annual or biennial herb that grows up to 1–2 meters in height. It has large, glossy green leaves with distinctive white veins and sharp spiny margins. The plant produces purple flower heads surrounded by spiny bracts and bears black to brown seeds, which are the primary source of the medicinal compound silymarin.

The plant is native to the Mediterranean region and is widely cultivated in Europe, Asia, North America, South America, and Australia.

Parts Used

Seeds (fruits), leaves, and roots; however, the seeds are the principal medicinal part.

Major Phytoconstituents

Silymarin (silybin, silydianin, silychristin), taxifolin, quercetin, apigenin, kaempferol, flavonoids, phenolic compounds, and fixed oils.

Traditional Uses

Milk thistle has traditionally been used for the treatment of liver diseases, jaundice, gallbladder disorders, digestive problems, and to protect the liver from toxins.

Pharmacological Activities

- Hepatoprotective
- Antioxidant
- Anti-Inflammatory
- Neuroprotective
- Anticancer
- Antidiabetic
- Cardioprotective
- Nephroprotective

Materials And Methods

Software Used

The following software and online tools were used for molecular docking studies:

- ❖ Discovery Studio Visualizer – Visualization, cleaning, and preparation of protein structures.
- ❖ Auto Dock 4.2 – Molecular docking and binding affinity prediction.
- ❖ Chem Sketch/Chem Draw – Drawing and editing phytochemical structures.
- ❖ PubChem Database – Retrieval of phytochemical structures and SMILES notations.
- ❖ CACTUS Online SMILES Translator (NCI/CADD) – Conversion of SMILES into three-dimensional (.pdb) structures.
- ❖ MGL Tools (Auto Dock Tools & Auto Grid) – Preparation of protein and ligand files, addition of Gasteiger charges, grid generation, and conversion to PDBQT format.
- ❖ Cygwin Terminal – Execution of AutoGrid and AutoDock command-line docking processes.
- ❖ Discovery Studio Visualizer – Analysis and visualization of protein–ligand interactions after docking.

Selection of Medicinal Plant

Silybum marianum (L.) Gaertn. (Family: Asteraceae), commonly known as Milk thistle, was selected for the present study based on its extensive traditional and medicinal use. The plant has been traditionally employed for the management of liver disorders, inflammation, oxidative stress, and metabolic disorders. Its major bioactive constituents, particularly silymarin and silybin, have demonstrated hepatoprotective, antioxidant, anti-inflammatory, and cytoprotective properties. Recent pharmacological studies have also reported its potential in neuroprotective, anti-inflammatory, antioxidant, and anti-stroke activities, making it a suitable candidate for molecular docking studies

Collection of Phytochemical Data

Reported phytochemical constituents of Milk Thistle were collected from published research articles, phytochemical databases, and review literature. Information was obtained from the IMPPAT (Indian Medicinal Plants, Phytochemistry and Therapeutics) database and PubChem database. Major phytoconstituents selected for molecular docking studies included.

Selection of Target Proteins

Based on their established role in the pathogenesis of Milk Thistle. Protein structures were retrieved from the RCSB Protein Data Bank (PDB). The selected proteins satisfied the following criteria:

- ❖ Availability of high-resolution crystal structures.
- ❖ Direct involvement in stroke-related pathological pathways.

❖ Presence of a well-defined active binding site suitable for molecular docking.

Ligand Preparation

The three-dimensional structures of the selected phytochemicals were obtained by retrieving their SMILES notations from the PubChem database. The SMILES strings were converted into PDB format using the CACTUS Online SMILES Translator (NCI/CADD). The generated structures were imported into AutoDock Tools (MGL Tools), where hydrogen atoms and Gasteiger charges were added, rotatable bonds (torsions) were assigned, and the ligands were finally saved in PDBQT format for molecular docking studies.

Target Protein Preparation

The selected proteins from specific organism are downloaded in pdb format. Open it in discovery bio visualizer and delete the unwanted chains. The unwanted chains and atoms which includes ligand group, repeated chains and non-active sites. Then the finalized protein molecule saved in pdb format. In Auto Dock Tools, add polar hydrogens then Add Kollman charges and Save as PDBQT (rigid macromolecule).

Molecular Docking Study

a) Grid Generation (Auto Grid)

- Load both protein and ligand.
- Set grid box around active site, set default grid parameters
- Save in .gpf file format.
- Run autogrid4 command in Cygwin
- Results in Producing grid map files

b) Docking (AutoDock)

- Load both protein and ligand
- Use Lamarckian Genetic Algorithm (LGA)
- Save .dpf file
- Run autodock4 command in Cygwin
- Results in output file: dlg (docking log file)

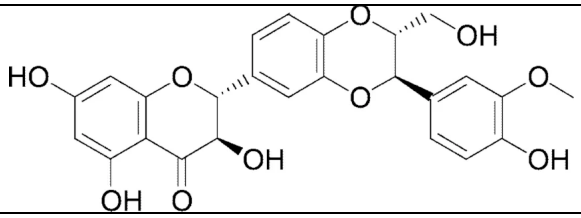
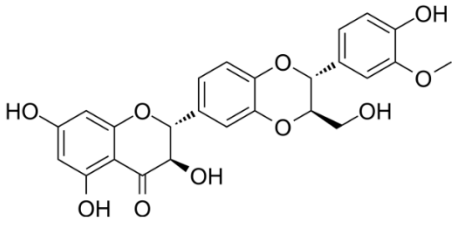
Interaction Visualization and Result Analysis

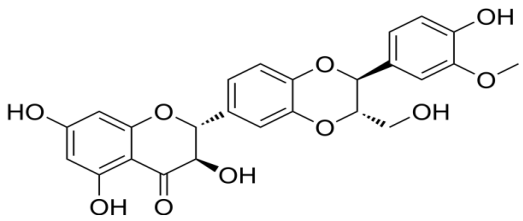
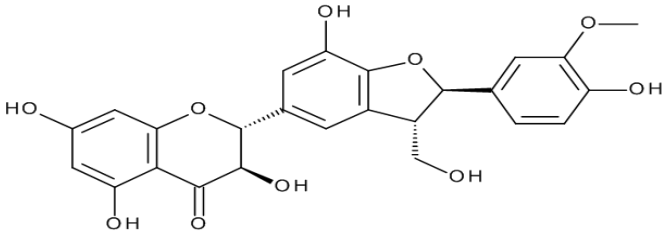
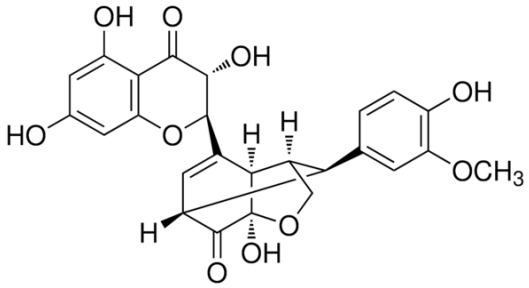
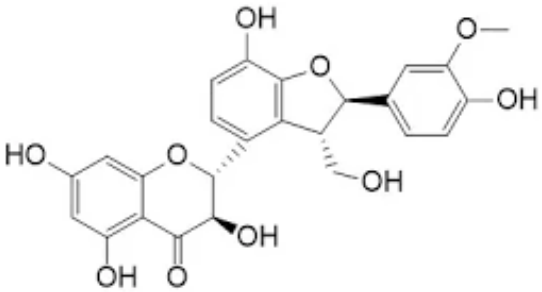
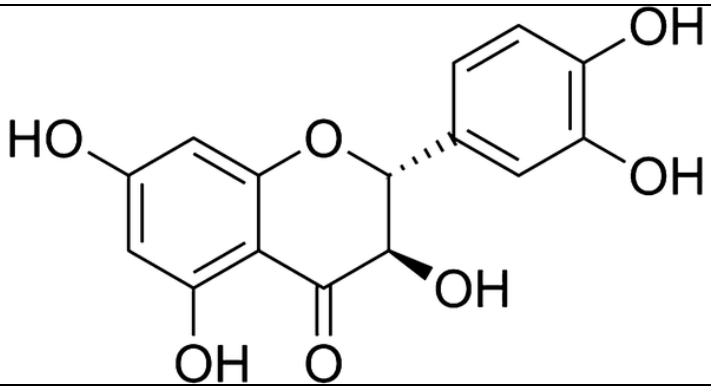
The resultant .dlg file opened for the docking score. And also open that file in AutoDock tool and load protein in order to visualize the interactions between protein and ligand.

Data Interpretation

Docking scores and interaction patterns of the selected Milk Thistle (*Silybum marianum*) phytoconstituents were compared to evaluate their binding efficiency toward the selected target proteins associated with hepatoprotective, cardioprotective, antioxidant, anti-inflammatory, nephroprotective, anticancer, and neuroprotective activities. Phytochemicals exhibiting strong binding affinity and favorable protein–ligand interactions were considered potential lead compounds for further pharmacological investigation and drug development.

Table 1: Structure of Phytochemical constituents

S. NO	CHEMICAL CLASS	CHEMICAL CONSTITUENTS	STRUCTURE
1.	Flavonolignan	Silybin	
2.	Flavonolignan	Isosilybin A	

3.	Flavonolignan	Isosilybin B	
4.	Flavonolignan	Silychristin	
5.	Flavonolignan	Silydianin	
6.	Flavonolignan	Isosilychristin	
7.	Flavonoid	Taxifolin	

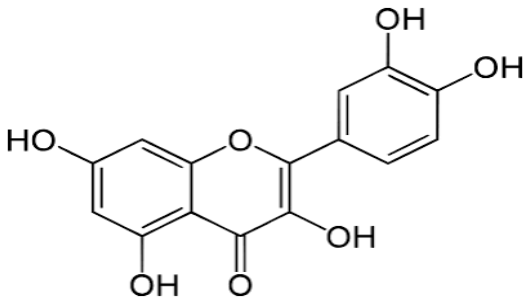
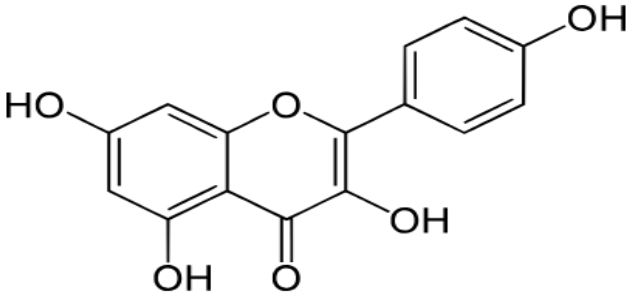
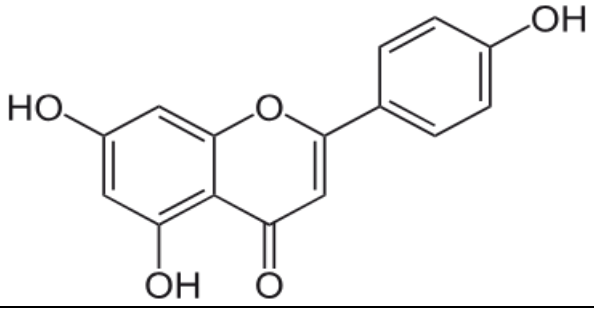
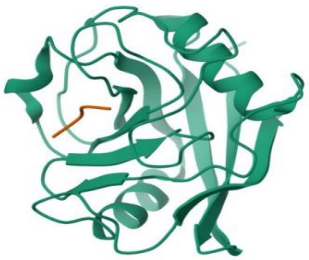
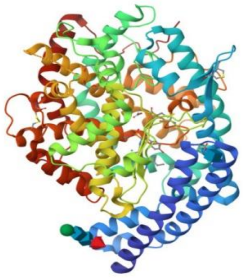
8.	Flavonoid	Quercetin	
9.	Flavonoid	Kaempferol	
10.	Flavonoid	Apigenin	

Table 2: structure of Target Protein.

Sno	Pharmacological Activities	Targeted protein with PBDID		Structure of targeted protein
1.	Hepatoprotective	NRF2	8HZ8	
2.	Cardioprotective	ACE	4BZR	

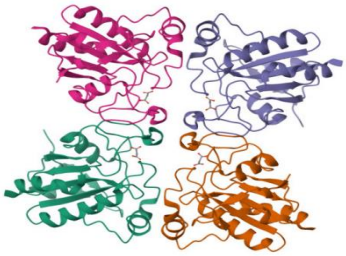
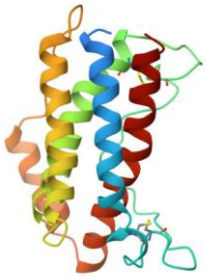
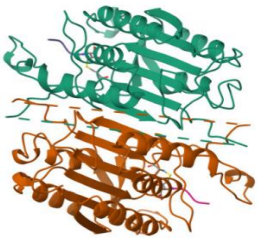
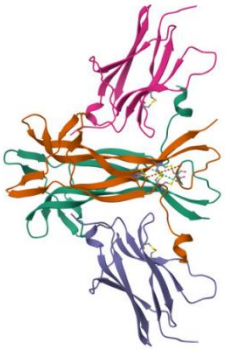
3.	Antioxidant	SOD1	2F8A	
4.	Anti-inflammatory	IL-6	1IL6	
5.	Nephroprotective	KIM-1	3LN3	
6.	Anticancer	Capase-3	1CP3	
7.	Neuroprotective	AchE	1WWW	

Table 3: Docking scores for Hepatoprotective.

S. No	Ligand name (phytochemicals)	Protein ID	8HZ8
		Binding Energy	Inhibition constant
1	Silybin	-4.57	450.41µM
2	Isosilybin A	-4.72	345.57µM
3	Isosilybin B	-4.90	254.64µM
4	Silychristin	-4.62	411.79µM
5	Silydianin	-3.85	1.52µM
6	Isosilychristin	-3.92	1.33µM
7	Taxifolin	-4.80	305.00µM
8	Quercetin	-4.25	763.89µM
9	Kaempferol	-3.74	1.80µM
10	Apigenin	-5.96	42.69µM

Table 4: Docking scores for Cardioprotective.

S. No	Ligand name (phytochemicals)	Protein ID	4BZR
		Binding Energy	Inhibition constant
1	Silybin	-7.01	7.31µM
2	Isosilybin A	-5.14	171.21µM
3	Isosilybin B	-6.36	21.90µM
4	Silychristin	-6.56	15.43µM
5	Silydianin	-7.35	1.76µM
6	Isosilychristin	-7.27	4.72µM
7	Taxifolin	-5.61	76.87µM
8	Quercetin	-6.41	20.05µM
9	Kaempferol	-6.79	10.62µM
10	Apigenin	-7.08	6.43µM

Table 5: Docking scores for Antioxidant.

S. No	Ligand name (phytochemicals)	Protein ID	2F8A
		Binding Energy	Inhibition constant
1	Silybin	-4.90	256.50µM
2	Isosilybin A	-5.05	197.38µM
3	Isosilybin B	-1.70	57.09µM
4	Silychristin	-7.17	5.52µM
5	Silydianin	-5.31	128.76µM
6	Isosilychristin	-3.26	4.05µM
7	Taxifolin	-4.30	200.34µM
8	Quercetin	-4.94	238.41µM
9	Kaempferol	-3.23	4.50µM
10	Apigenin	-5.25	120.67µM

Table 6: Docking scores for Anti-inflammatory.

S. No	Ligand name (phytochemicals)	Protein ID	11L6
		Binding Energy	Inhibition constant
1	Silybin	-1.91	39.72µM
2	Isosilybin A	-0.51	423.00µM
3	Isosilybin B	-4.86	272.61µM
4	Silychristin	-2.64	11.55µM
5	Silydianin	-4.06	1.06µM
6	Isosilychristin	-5.44	103.44µM
7	Taxifolin	-5.91	46.75µM
8	Quercetin	-6.22	27.36µM
9	Kaempferol	-6.26	25.72µM

10	Apigenin	-6.67	12.98µM

Table 7: Docking scores for Nephroprotective.

S. No	Ligand name (phytochemicals)	Protein ID	3IN3
		Binding Energy	Inhibition constant
1	Silybin	-5.17	163.26µM
2	Isosilybin A	-3.83	1.55µM
3	Isosilybin B	-4.78	315.92µM
4	Silychristin	-3.73	1.86µM
5	Silydianin	-4.94	240.18µM
6	Isosilychristin	-3.69	1.69µM
7	Taxifolin	-4.88	267.02µM
8	Quercetin	-5.20	153.01µM
9	Kaempferol	-5.35	120.40µM
10	Apigenin	-5.46	98.81µM

Table 8: Docking scores for Anticancer.

S. No	Ligand name (phytochemicals)	Protein ID	1CP3
		Binding Energy	Inhibition constant
1	Silybin	-5.56	83.70µM
2	Isosilybin A	-5.39	111.62µM
3	Isosilybin B	-5.23	147.75µM
4	Silychristin	-5.95	43.25µM
5	Silydianin	-7.38	3.93µM
6	Isosilychristin	-4.48	517.34µM
7	Taxifolin	-6.22	27.39µM
8	Quercetin	-5.78	58.40µM
9	Kaempferol	-6.28	24.88µM
10	Apigenin	-4.54	469.50µM

Table 9: Docking scores for Neuroprotective.

S. No	Ligand name (phytochemicals)	Protein ID	1WWW
		Binding Energy	Inhibition constant
1	Silybin	-5.25	202.34µM
2	Isosilybin A	-8.63	468.24µM
3	Isosilybin B	-9.28	158.61µM
4	Silychristin	-9.30	153.07µM
5	Silydianin	-5.06	196.15µM
6	Isosilychristin	-6.86	9.30µM
7	Taxifolin	-4.93	242.06µM
8	Quercetin	-8.77	371.60µM
9	Kaempferol	-8.21	954.15µM
10	Apigenin	-8.79	360.45µM

Table 10: ADME studies of the phytochemical constituents.

Phytochemicals	Molecular weight (g/mol)	HBA	HBD	Log p	GI absorption	BBB permeability	Skin permeability (cm/s)	Bio availability	Lipinski rule
Silybin	482.441	10	5	2.3627	Low	No	-2.735	0.55	Yes
Isosilybin A	482.441	10	5	2.3627	Low	No	-2.735	0.55	Yes

Isosilybin B	482.441	10	5	2.3637	Low	No	-2.735	0.55	Yes
Silychristin	482.441	10	6	2.4045	Low	No	-2.735	0.55	Yes
Silydianin	482.441	10	5	0.9905	Low	No	-2.735	0.55	Yes
Isosilychristin	482.442	10	6	2.4045	Low	No	-2.735	0.55	Yes
Taxifolin	304.254	7	5	1.1863	High	No	-2.735	0.55	Yes
Quercetin	302.238	7	5	1.988	High	No	-2.735	0.55	Yes
Kaempferol	286.239	6	4	2.2824	High	No	-2.735	0.55	Yes
Apigenin	270.24	5	3	2.5768	High	No	-2.735	0.55	Yes

Table 11: Toxicity studies of the phytochemical constituents.

Phytochemicals	Total clearance (ml/min/kg)	AMES toxicity	Max. tolerated dose (mg/kg/day)	Hepatic toxicity	Skin sensitization	Minnow toxicity (log mm)
Silybin	-0.103	No	0.65	No	No	2.543
Isosilybin A	-0.106	No	0.658	No	No	2.297
Isosilybin B	-0.005	No	0.761	No	No	1.105
Silychristin	-0.169	No	0.612	No	No	3.66
Silydianin	-0.065	No	0.297	No	No	2.452
Isosilychristin	-0.121	No	0.549	No	No	4.598
Taxifolin	-0.078	No	0.345	No	No	4.688
Quercetin	0.407	No	0.499	No	No	3.721
Kaempferol	0.591	Yes	0.82	No	No	1.082
Apigenin	0.566	No	0.328	No	No	2.432

Figure 2: Docking visuals of Hepatoprotective activity
Taxifolin with 8HZ8Apigenin with 8HZ8

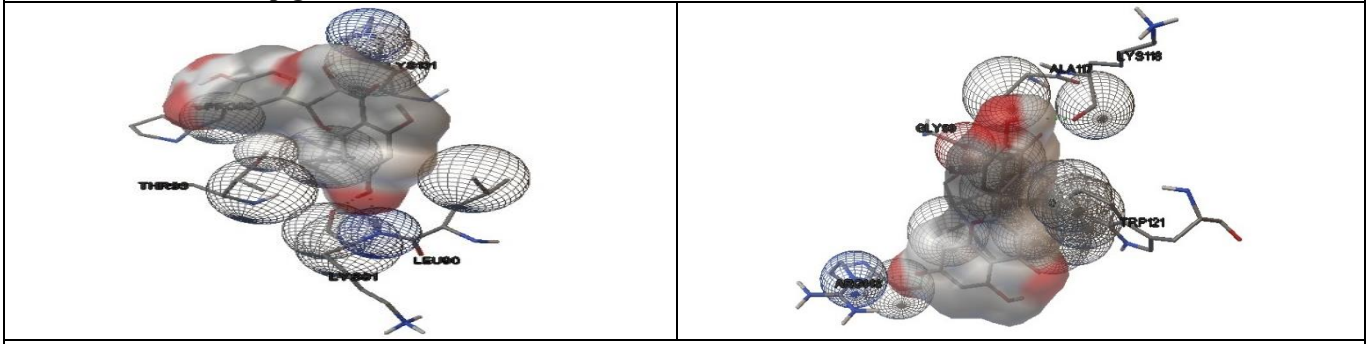


Figure 3: Docking visuals of Cardio protective activity.
Silydianin with 4BZRApigenin with 4BZR

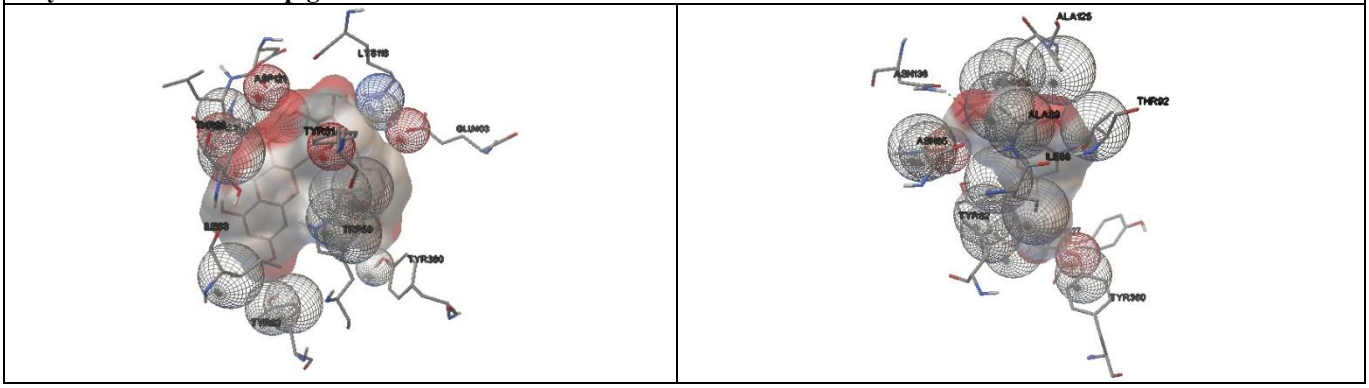
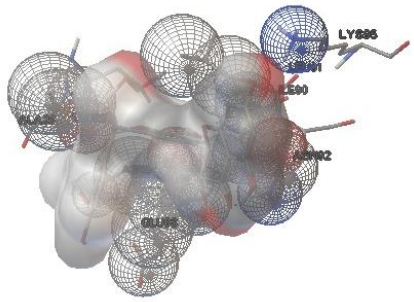


Figure 4: Docking visuals of Antioxidantactivity.
Silychristin with 2F8AIsosilybin with 2F8A



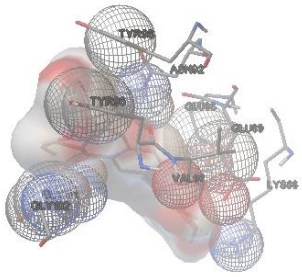
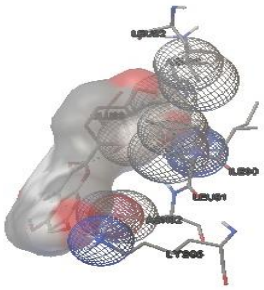


Figure 5: Docking visuals of Anti-inflammatory activity.
Silybin with 1IL6Isosilybin A with 1IL6



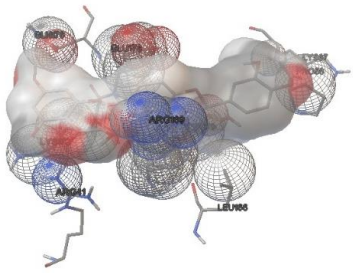
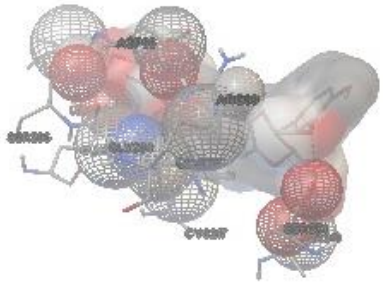


Figure 6: Docking visuals of Nephroprotective activity.
Silybin with 31N3Silychristin with 31N3



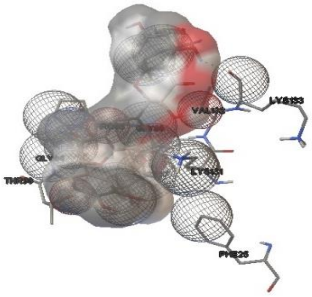
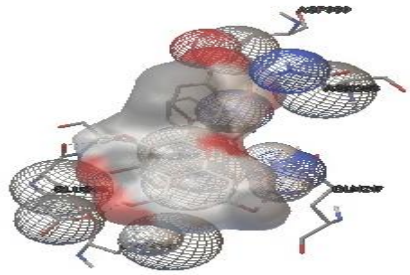


Figure 7: Docking visuals of Anticanceractivity.
Quercetin with 1CP3 Kaempferol with 1CP3



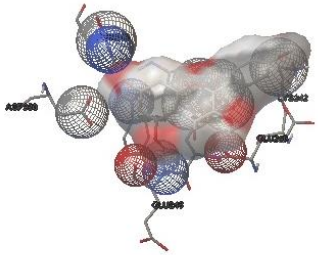
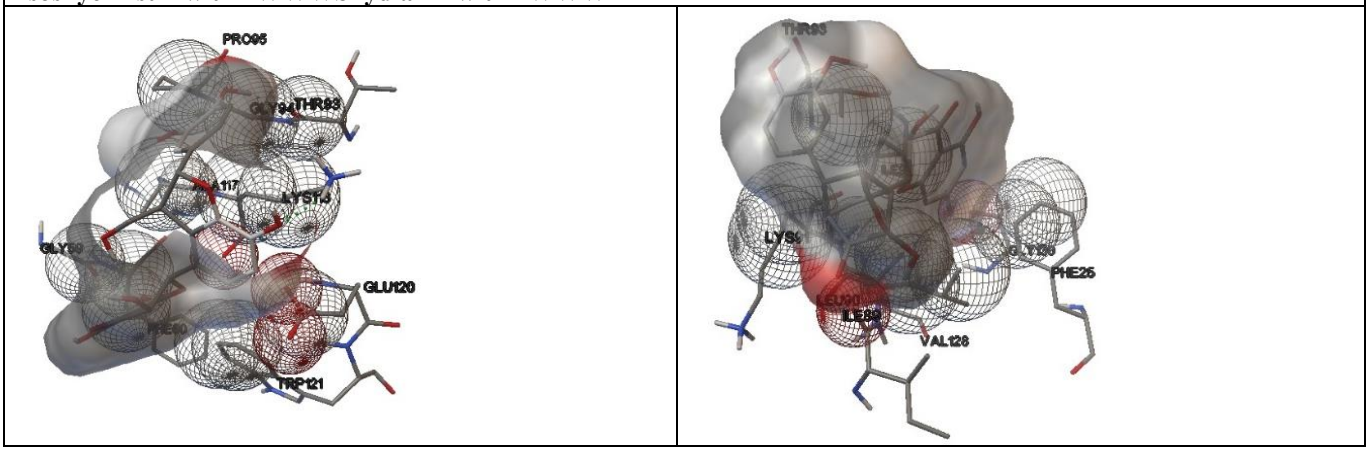


Figure 8: Docking visuals of Neuroprotective activity.
Isosilychristin with 1WWWSilydianin with 1WWW



RESULTS AND DISCUSSION

Hepatoprotective Activity

This protein is associated with liver protection and maintenance of normal liver function. It is involved in preventing liver cell damage caused by oxidative stress and toxic substances.

Molecular docking of the selected phytochemicals against the hepatoprotective target protein (PDB ID: 8HZ8) revealed that all compounds exhibited moderate binding affinities. Among them, Apigenin showed the strongest interaction with a binding energy of −5.96 kcal/mol, indicating better binding stability than the other phytochemicals. Isosilybin B and Taxifolin also demonstrated favorable binding scores, suggesting that these compounds may contribute to the hepatoprotective potential of Milk Thistle.

Cardioprotective Activity

This protein plays an important role in maintaining normal heart function and protecting cardiac tissues. It is commonly used to evaluate compounds with cardioprotective potential.

The docking study against the cardioprotective target protein (PDB ID: 4BZR) showed promising interactions for all selected phytochemicals. Silydianin exhibited the highest binding affinity (−7.35 kcal/mol), followed closely by Isosilychristin and Apigenin. These strong docking scores indicate that these phytoconstituents possess good binding stability and may have significant cardioprotective activity

Antioxidant Activity

This protein is involved in the cellular antioxidant defense system by reducing oxidative stress. It helps protect cells from damage caused by reactive oxygen species (ROS).

Docking analysis against the antioxidant target protein (PDB ID: 2F8A) demonstrated that Silychristin had the best binding affinity (−7.17 kcal/mol). Silydianin, Isosilybin A, and Quercetin also showed favorable interactions with the target protein. These findings suggest that the antioxidant activity of Milk Thistle is mainly associated with its flavonolignan constituents.

Anti-Inflammatory Activity

This protein is associated with inflammatory signaling pathways. Inhibiting its activity may reduce inflammation and prevent tissue damage.

The anti-inflammatory docking study using protein 11L6 revealed that Apigenin showed the strongest binding affinity (−6.67 kcal/mol), followed by Kaempferol and Quercetin. These phytochemicals formed stable interactions with the target protein, indicating their potential to inhibit inflammatory pathways. The results support the traditional use of Milk Thistle in inflammatory disorders.

Nephroprotective Activity

Nephroprotective activity is the ability to protect the kidneys from toxic substances and oxidative stress. It helps preserve normal kidney structure and function.
Among all the phytochemicals docked against the nephroprotective target protein (listed as protein ID 3IN3 in the table), Apigenin exhibited the highest binding affinity (−5.46 kcal/mol), followed by Kaempferol and Quercetin. These results indicate that flavonoids present in Milk Thistle may play an important role in protecting renal tissues against oxidative stress and cellular injury.

Anticancer Activity

Anticancer activity is the ability to inhibit the growth and spread of cancer cells. It promotes cancer cell death and prevents tumour progression.

Docking studies against the anticancer target protein (PDB ID: 1CP3) showed that Silydianin demonstrated the strongest interaction with a binding

energy of −7.38 kcal/mol. Kaempferol, Taxifolin, and Silychristin also exhibited good binding affinities, suggesting that these phytochemicals may interfere with cancer-related molecular targets and possess potential anticancer properties.

Neuroprotective Activity

Neuroprotective activity is the ability to protect nerve cells from degeneration and injury. It helps maintain brain function and reduces the risk of neurological disorders.

The molecular docking results against the neuroprotective target protein (PDB ID: 1WWW) indicated that Silychristin had the highest binding affinity (−9.30 kcal/mol), followed by Isosilybin B, Apigenin, and Quercetin. These strong interactions suggest excellent binding stability with the target protein and support the potential neuroprotective role of Milk Thistle phytoconstituents in the management of neurological disorders.

ADMET Analysis of Milk Thistle

For the analysis of the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the phytoconstituents of Silybum marianum (Milk Thistle), the selected compounds were collected from the IMPPAT and PubChem databases. Their SMILES notations were analyzed using the Swiss ADME web server to predict physicochemical properties, absorption, distribution, metabolism, drug-likeness, and bioavailability. Toxicity and excretion parameters were further evaluated using the pk CSM online server. The ADMET profile of each phytoconstituent was assessed based on molecular weight (MW), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), lipophilicity (LogP), bioavailability score, gastrointestinal (GI) absorption, blood–brain barrier (BBB) permeability, skin permeability, Lipinski's rule of five, total clearance, AMES toxicity, hepatotoxicity, and skin sensitization.

The phytoconstituents of Silybum marianum, including Silybin, Isosilybin A, Isosilybin B, Silychristin, Silydianin, Isosilychristin, Taxifolin, Quercetin, Kaempferol, and Apigenin, exhibited diverse ADMET characteristics. The flavonoids Taxifolin, Quercetin, Kaempferol, and Apigenin demonstrated high gastrointestinal absorption, whereas the flavonolignans generally showed low GI absorption. All compounds complied with Lipinski's rule of five and possessed a bioavailability score of 0.55, indicating favorable drug-like properties. None of the phytoconstituents were predicted to cross the blood–brain barrier, while all showed acceptable skin permeability values.

Toxicity prediction revealed that the majority of the selected phytoconstituents were non-mutagenic (AMES negative), non-hepatotoxic, and non-skin sensitizing. Kaempferol was the only compound predicted to be AMES positive, while the remaining phytoconstituents exhibited acceptable safety profiles with favorable total clearance values. Overall, the phytoconstituents of Silybum marianum demonstrated promising ADMET characteristics, supporting their potential as drug-like molecules for further development, particularly in hepatoprotective, antioxidant, anti-inflammatory, cardioprotective, nephroprotective, anticancer, and neuroprotective applications

CONCLUSION

The present molecular docking study demonstrated that the major phytoconstituents of Milk Thistle **(***Silybum marianum***)**, including Silybin, Isosilybin A, Isosilybin B, Silychristin, Silydianin, Isosilychristin, Taxifolin, Quercetin, Kaempferol, and Apigenin**,** exhibited favorable interactions with target proteins associated with hepatoprotective, cardioprotective, antioxidant, anti-inflammatory, nephroprotective, anticancer, and neuroprotective activities. Among the tested compounds, Apigenin, Silydianin, and Silychristin showed the strongest binding affinities against several target proteins, indicating promising therapeutic potential.

The ADME and toxicity predictions suggested that the selected phytochemicals possess acceptable drug-likeness and safety profiles, with no predicted AMES toxicity, supporting their potential as drug candidates. Overall, the findings indicate that Milk Thistle is a valuable medicinal plant with multiple pharmacological activities**,** and its phytoconstituents may serve as promising lead molecules for future drug discovery. However, further in vitro, in vivo, and clinical studies are required to validate these computational findings and confirm their therapeutic efficacy.

REFERENCES

1. Liver Tox: Clinical and Research Information on Drug-Induced Liver Injury, Milk Thistle, *National Institute of Diabetes and Digestive and Kidney Diseases*, Bethesda (MD), 2020.
2. Nawaz A, Zaib S, Khan I, Ahmed A, Shahzadi K, Riaz H, Silybum marianum: An overview of its phytochemistry and pharmacological activities with emphasis on potential anticancer properties, *Anticancer Agents in Medicinal Chemistry*, 23(13), 2023, 1519–1534.
3. Bijak M, Silybin, a major bioactive component of milk thistle (Silybum marianum L. Gaertn.)—chemistry, bioavailability, and metabolism, *Molecules*, 22(11), 2017.

4. Dwyer JT, Coates PM, Smith MJ, Dietary supplements: Regulatory challenges and research resources, *Nutrients*, 10(1), 2018.
5. Williams R, Global challenges in liver disease, *Hepatology*, 44(3), 2006, 521–526.
6. Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S, Prevalence of and risk factors for non-alcoholic fatty liver disease: The Dionysos nutrition and liver study, *Hepatology*, 42(1), 2005, 44–52.
7. Velussi M, Cernigoi AM, De Monte A, Dapas F, Caffau C, Zilli M, Long-term (12 months) treatment with an antioxidant drug (silymarin) is effective on hyperinsulinemia, exogenous insulin needs and malondialdehyde levels in cirrhotic diabetic patients, *Journal of Hepatology*, 26(4), 1997, 871–879.
8. Hajiaghamohammadi AA, Ziaee A, Oveisi S, Masroor H, Effects of metformin, pioglitazone, and silymarin treatment on non-alcoholic fatty liver disease: A randomized controlled pilot study, *Hepatitis Monthly*, 12(8), 2012, e6099.
9. Kazazis CE, Evangelopoulos AA, Kollas A, Vallianou NG, The therapeutic potential of milk thistle in diabetes, *Review of Diabetic Studies*, 11(2), 2014, 167–174.
10. Huseini HF, Larijani B, Heshmat R, Fakhrzadeh H, Radjabipour B, Toliat T, Raza M, The efficacy of Silybum marianum (L.) Gaertn. (silymarin) in the treatment of type II diabetes: A randomized, double-blind, placebo-controlled, clinical trial, *Phytotherapy Research*, 20(12), 2006, 1036–1039.
11. Loguercio C, Federico A, Trappoliere M, Tuccillo C, de Sio I, Di Leva A, Niosi M, D'Auria MV, Capasso R, Del Vecchio Blanco C, Real Sud Group, The effect of a silybin-vitamin E-phospholipid complex on non-alcoholic fatty liver disease: A pilot study, *Digestive Diseases and Sciences*, 52(9), 2007, 2387–2395.
12. Hruby K, Csomos G, Fuhrmann M, Thaler H, Chemotherapy of Amanita phalloides poisoning with intravenous silibinin, *Human Toxicology*, 2(2), 1983, 183–195.
13. Rahnama S, Tehrankhah ZM, Mohajerani F, Mohammadi FS, Yeganeh ZY, Najafi F, Babashah S, Sadeghizadeh M, Milk thistle nano-micelle formulation promotes cell cycle arrest and apoptosis in hepatocellular carcinoma cells through modulating miR-155-3p/SOCS2/PHLDA1 signaling axis, *BMC Complementary Medicine and Therapies*, 23(1), 2023, 337.
14. Jo AI, Kim MM, Silibinin inhibits cell invasion through the inhibition of MMPs, p-p38, and IL-1β in human fibrosarcoma cells, *Frontiers in Bioscience (Landmark Edition)*, 28(4), 2023, 64.
15. Iqbal MA, Chattopadhyay S, Siddiqui FA, Ur Rehman A, Siddiqui S, Prakasam G, Khan A, Sultana S, Bamezai RN, Silibinin induces metabolic crisis in triple-negative breast cancer cells by modulating EGFR-MYC-TXNIP axis: Potential therapeutic implications, *The FEBS Journal*, 288(2), 2021, 471–485.
16. Kawaguchi-Suzuki M, Frye RF, Zhu HJ, Brinda BJ, Chavin KD, Bernstein HJ, Markowitz JS, The effects of milk thistle (Silybum marianum) on human cytochrome P450 activity, *Drug Metabolism and Disposition*, 42(10), 2014, 1611–1616.
17. Loguercio C, Festi D, Silybin and the liver: From basic research to clinical practice, *World Journal of Gastroenterology*, 17(18), 2011, 2288–2301.
18. Abenavoli L, Bellentani S, Milk thistle to treat non-alcoholic fatty liver disease: Dream or reality?, *Expert Review of Gastroenterology & Hepatology*, 7(8), 2013, 677–679.

Cite this article:
Dr. Saravanan C, Janani S. In-Silico Evaluation of Silybum Marianum Phytoconstituents for Hepatoprotective Activity Using Molecular Docking. *International Journal of Pharmaceutical Research & Analysis,* 16(2), 2026, 118-131.