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Investigation of anti-inflammatory and Anti-oxidant effects of physiologically attainable Quercetin concentrations through in-silico studies by *Ficus religiosa* seeds Extract

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Abstract
Background: Peepal (*Ficus religiosa*) has been well recognized in traditional medicine because of the involvement of therapeutic properties in Ayurvedic medicine known as 'World Tree' or the 'Tree of Life". Its seeds are believed to purify blood and promote overall health. Quercetin, a flavonoids present in the seeds, is known for its antioxidant and anti-inflammatory properties. **Aim:** This study presented the extraction and bioactive evaluation of the seeds of *Ficus religiosa* in relation to their biological activity, and focuses on the antioxidant and anti-inflammatory activity contributing to blood purification.
Method: The bioactive compounds isolated from seaweeds were treated using in silico data (molecular docking and some computer models) to predict their interactions with significant enzymes and proteins related with oxidative stress and inflammation. The antioxidant activity was determined according to the ability of the compounds to remove free radicals, seeds of *Ficus religiosa* was extracted by Maceration and Soxhlation using Ethanol and Water as solvents. The extracts of the seeds were tested for the presence of phytochemicals through phytochemical screening. The Blood Purification of the seeds were evaluated by using Analytical methods HPTLC (High Performance Thin Layer Chromatography for the presences of Quercetin
Results: As evident from the experimental data the ethanol extract showed the presence of Quercetin through HPTLC (High Performance or Presuure Thin Layer Chromatography and the In silico studies are evident for the binding capacity of Anti-Oxidant Activity as 74% and Anti In inflammatory Activity as 72% which can a scope for future determination.
Conclusion: The current study was notably focused on exploring new bioactive compounds that could be beneficial in the development of natural medicines targeting blood purification and its associated health benefits.

Keywords: *Ficus religiosa*, ayurvedic system, Quercetin, anti-inflammatory activity, anti-oxidant activity, blood purification

1. Introduction
The word "Ayurveda" comes from the word 'Ayur' meaning 'Life' and the word 'Veda' meaning' to 'Know'. Ayurveda means "the science of life". The World Health Organization (WHO) evaluates that 80% of the world population, currently using herbal medicine for some aspect of primary health care. Computer-Aided Drug Design (CADD) refers to the application of computational techniques and tools to discover, develop, and analyze new therapeutic compounds. CADD provides a cost-effective and time-efficient alternative to traditional trial-and-error laboratory approaches. In silico studies refer to research and experiments carried out using computational techniques and computer-based models. The term "in silico" is derived from the Latin word for silicon, which is the primary material in computer chips. Unlike traditional *in vitro* (in the lab) and *in vivo* (in living organisms) methods, in silico approaches use software programs, algorithms, and databases to simulate biological processes. Targets for blood purification can cover a wide spectrum of molecules, varying in size, polarity, and chemical and biological properties. Accordingly, blood purification may be performed with many different techniques. In principle, the basis of different therapeutic approaches lies in bioseparation science. It is very probable that a number of herbal remedies, the constituents of which are still unknown, will be shown to contain active flavonoids. The group is known for its anti-inflammatory and anti-allergic effects, antithrombitic and vasoprotective properties,

and inhibition of tumor promotion as well as protecting the gastric mucosa. Many flavonoids-containing plants are diuretics or antispasmodics and some flavonoids have antitumor, antifungal and antibacterial properties as well as antihepatotoxic activity. This study reports the quantitative estimation of quercetin in peepal tree seeds using an HPTLC method. The main objective of the present study investigated the possible anti-inflammatory effects of physiologically attainable quercetin concentrations through in silico studies for blood purification by selected plant *Ficus*

Fig 1: *religiosa* Twig and seeds

2. Plant Profile

Table 1: Taxonomy of *Ficus religiose*

Botanical Classification of Ficus *religiosa*		**Vernacular Names**	
Kingdom	*Plantae*	Sanskrit	Pippala
Subkingdom	*Viridaeplantae*	Hindi	Pipala, Pipal
Phylum	*Tracheophyta*	Telugu	Ravichettu
Subphylum	*Spermatophytina*	Tamil	Ashwarthan, Arasamaram
Class	*Magnoliopsida*	Guajarati	Piplo, Jari
Subclass	*Dilleniidae.*	Bengali	Asvattha, Ashud
Order	*Urticales*	*Marathi*	Pipal, Pimpal
Family	*Moraceae*	Punjabi	Pipal, Pippal
Division	*Magnoliophyta*	Oriya	Aswatha
Genus	*Ficus (FY-kus) Linnaeus*	Kannada	Basari, Ashvattha
Species	*Ficus religiosa*	English	Pipal tree

3. Plant Description

Ficus religiosa Linn (Moraceae) commonly known as 'Peepal tree' is a large widely branched tree with leathery, heart-shaped, long-tipped leaves on long slender petioles and purple fruits growing in pairs. Bears pairs of rounded, flat-topped green figs, to 1/2 inch (1.5 cm) across, ripening to purple with red dots. The trunk has smooth grey bark and with age this trunk becomes irregularly shaped. This big and old tree is of 30 m long. Phytochemical compounds such as Asparagine and tyrosine are the most abundant amino acids of the fruit pulp of F. religiosa.26 F. religiosa fruits contain a considerable amount of flavonoids namely kaempeferol, quercetin, and myricetin and other phenolic components. The seeds contain phytosterolin, β-sitosterol, and its glycoside, albuminoids, carbohydrate, fatty matter, coloring matter, caoutchoue 0.7 5.1%.

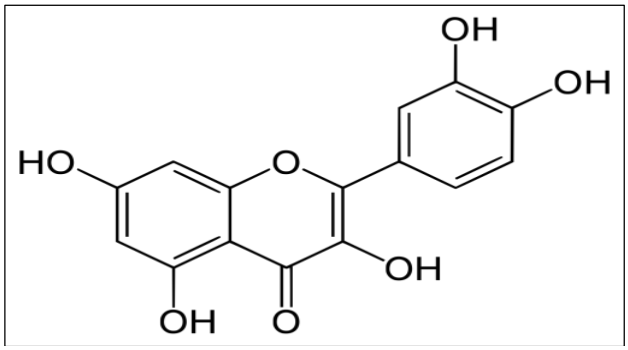

Fig 2: Quercetin Structure

4. Materials and Methods

The seeds of *Ficus religiosa* were collected from the Kurnool district Andhrapradesh and Plant is authenticated at government institute of study in advanced education kurnool-518002, A.P., India by botanist.

Table 2: Authentication details of *Ficus religiosa*

Botanical Name	**Voucher No**	**Family**
Ficus religiosa	M328	*Moraceae.*

Table 3: List of Chemicals Used

Si.no	**Constituents**	**Quality**	**Manufacturer**
1	Crude Extract of *Ficus religiosa*	514gm	-
2	Ethanol	600ml	-
3	Distilled Water	1000ml	-
4	Marketed Quercetin (25mg/ml)	25mg	Madhu Agency Kurnool Andhrapradesh

5. Method of Extraction of *Ficus Religiosa*

Soxhlet extraction procedure is adopted for extraction of *Ficus religiosa* seed powder, A Soxhlet extraction is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. It was originally designed for a liquid from a solid material.

Procedure for Aqueous Extract of *Ficus Religiosa*

1. 180gms of the Ficus religiosa powder was taken in Soxhlet apparatus
2. To base of the Soxhlet tube a round bottomed flask was attached in which 180ml of distilled water and 420ml of ethanol was taken in 70:30 ratio
3. To the round bottomes flask porcelain pieces are added to avoid bumping
4. The crude drug was maintained in a Filter paper to for the pure form of extract
5. A cotton plug was placed inside the Soxhlet apparatus to avoid the passage of crude drug
6. Temperature of 80 degree was maintained using heating mantle
7. Continue water inlet and outlet was set up to the Soxhlet apparatus
8. Extraction was performed for 10 hours
9. After 10 hours aqueous extract was collected in the round bottomed flask
10. The other batch of the powder was performed through Maceration process

Procedure for Ethanol Extraction

1. 150grams of two batches of Ficus religiosa powder was placed in a stoppered container

2. Added 350ml of etanol and150ml of distilled water in 70:30 ratiomand allowed to stand for a period of at least 3-4 days with frequent agitation
3. Until soluble matter is dissolved
4. The mixture is then strained through filter paper and funnel
5. After 3-4 days non, aqueous extract was collected in the round bottomed flask
6. To get concentrated powder from the above extract.

Table 4: Aqueous and Ethanolic extract of *Ficus religiosa* seed powder

Ficus religiosa	Aqueous	Ethanol
100grams	30ml	70ml
200 grams	60ml	140ml
300grams	90ml	210ml

Fig 3: various steps Involved in Extraction of *Ficus religiosa* seed powder

6. Preliminary Phytochemical Screening of *Ficus religiosa* seed powder

A preliminary phytochemical screening confirmed flavonoids. The highest detection was for Quercetin - a well-known flavonoid with superior antioxidant and anti-inflammatory - and also the main extract bio constituent that was investigated. Quercetin was confirmed in the extract and quantified using laboratory analytical techniques, including High-Performance Thin Layer Chromatography (HPTLC). The seed extract blood purification potential was evaluated for antioxidant activity and anti-inflammatory through in silico molecular docking studies.

7. Experimental Procedure for Detection of Presence of Quercetin using HPTLC

Reagents and materials

1. Peepal tree seeds were collected during the month of January 2025. The standards of Quercetin were purchased from Madhu Agency Kurnnol, Andhrapradesh.
2. The TLC plates RP-18 F254 S (5×7.5 cm) were used without any pretreatment. All chemicals and solvents used were of analytical and HPLC grade.
3. Standard stock solutions of Quercetin (1 mg/ml) were prepared by dilution in methanol and passed through 0.45 Millipore filters.
4. A sample was prepared from a coarse powder of dried Peepal tree seeds and the powder (50 g) was refluxed with ethanol in a Soxhlet extractor for 72 h.
5. A known amount of the concentrated extract (200 mg) was taken and dissolved in methanol (10 ml) and then 1 ml of this solution was diluted to 10 ml and passed through a 0.45 µm Millipore filter.
6. HPTLC analysis A Camag HPTLC system equipped with an automatic TLC sampler with a UV cabinet and a twin trough glass tank were used.
7. The samples were applied using an automated TLC sampler in 5 mm bands at 10 mm from the X and Y axis and 5 mm spaces were left between adjacent bands.
8. Calibration and quantification Standard stock solutions of Quercetin (1 mg/ml) were diluted in methanol to obtain the working solutions (0.10 mg/ml) for Quercetin.
9. The obtained working standard solutions were then applied to RP-TLC plates to prepare four-point linear calibration curves.
10. Quercetin were both applied in volumes of 5, 10, 15, and 20 µl. For sample quantification, 5 µl Quercetin samples were applied to the plates as 5 mm bands at intervals of 5 mm.
11. The experiment was performed in triplicate. The TLC plates were developed in a Camag twin-trough glass tank which was pre-saturated with developing solvent (40:57:3, v/v/v) up to a height of 7 cm.
12. The composition of the developing solvent was selected from the method previously developed. After development, the plate was removed, dried and spots were visualized under UV light at 254 nm.
13. Quantitative evaluation of the plate was performed in the reflectance/absorbance mode at 254 nm under the following conditions: slit width (6×0.4 mm, Micro), scanning speed (20 mm/s) and data resolution (100 µmstep).
14. 14.The seed extract blood purification potential was evaluated for antioxidant activity and anti-inflammatory through in silico molecular docking studies were included

to examine the molecular interactions of Quercetin with a few target proteins such as Human Serum Albumin (HAS) involved in oxidative-stress and inflammation, supporting the blood purification act of therapeutic relevance.

8. Procedure for In Silico Studies Docking Methods for Anti-Inflammatory and Anti-Oxidant on Human Serum Albumin (HSA)

In Silico studies through molecular docking (Computer Aided Design) Computational Technique and the results we got through Anti Inflammatory and Anti-Oxidant on Human Serum Albumin (HSA)

Docking Method

1. Preparation of the Protein (Receptor)

- Obtained structure: Download from the Protein Data Bank (PDB).
- The receptor that is used is HISTAMINE, HUMAN SERUM ALBUMIN receptor
- Removed water molecules (unless needed).
- Deleted ligands or ions (unless they're part of the active site).
- Added polar hydrogens.
- Assign Kollman charges (in Auto Dock).
- Equalize charge for any residue

Saved the cleaned structure as PDBQT format

2. Prepared the Ligand

- Obtained the ligand structure (using PubChem).
- The selected ligand is quarecetin
- Optimized geometry and assign Gasteiger charges.
- Defined rotatable bonds.
- Saved the ligand as PDBQT.

3. Defined the Grid Box (Search Space)

- Set the center and dimensions of the grid box to encompass the active/binding site.
- This is where the docking simulation will search for binding conformations.

4. Run Docking

- Used AutoDock or AutoDock Vina:
- AutoDock uses a Lamarckian Genetic Algorithm.
- AutoDock Vina is faster and uses a different scoring function.

Input

- Receptor PDBQT
- Ligand PDBQT
- Grid configuration (in a config file for Vina)

Output

- Binding poses (conformations)
- Binding affinity (in kcal/mol)

5. Analysis

Analysis is done by the RSMD values, the binding capacity of the receptor and ligand reported in results.

9. Quercetin Binding Capacity for Anti Inflammatory Activity

Queractin binding capacity with histamine for the anti-inflammatory action of the molecule

- **Binding capacity:** Weak bonding
- **Receptor/Histamine code:** pdb_00007f61

Ligand/ quercetin code: 5280343

- It is the binding capacity of Quercetin molecule for its Anti-Inflammatory Activity
- **It has high binding capacity on TRP:** 402 and LEU: 401 on Azole of Quercetin showed an increase in energy values was more compatible with the receptor than the rest of the molecules.

10. Quercetin Binding Capacity for Anti-Oxidant Activity

Queractin binding capacity with Albumin for the anti oxidant action of the molecule

- **Receptor / Albumin:** pdb_00001a06
- **Ligand/ Quercetin code:** 5280343

It is the binding capacity of Quercetin molecule for its Anti-Oxidant Activity

It has high binding capacity on GLN: 459 and GLU: 425 on Hydroxyl groups and Carboxylic groups of Quercetin showed an increase in energy values was more compatible with the receptor than the rest of the molecules. Analysis was done by the RSMD values, the binding capacity of the receptor and ligand reported in results.

11. Procedure for Hptlc Results for Peepal Tree Seeds on The Presence of Quercetin

1. To estimate Quercetin in Peepal tree seeds TLC was performed on RP-TLC plates using the solvent system methanol-water-formic acid (40:57:3, v/v/v), which produced good separation with Rf values of 0.07 (Quercetin) for the ethanolic extract and reference compounds.
2. The ethanolic extract was able to resolve 6 compounds in the developing solvent system. The identity of the bands of Quercetin in the ethanolic extract was confirmed by comparing the UV-Vis absorption spectra with those of standards using a CAMAG TLC scanner 3.
3. The four-point linear calibration curves of both compounds were found to be linear over the range 500-2000 ng.
4. Good recoveries were obtained following enrichment of the sample at two different concentrations. The results showed that the percentage recoveries after sample processing and application were in the range of 100.12% to 100.21% (Quercetin).

12. Results and Discussion

Table 5: Preliminary phytochemical screening.

SI.No	Tests	Ethanolic Extract	Aqueous extract
1	Carbohydrates	+	+
2	Alkaloids	+	+
3	Volatile Oils	+	+
4	Tannins	+	+
5	Resins	_	_
6	Flavonoids	+	+
7	Glycoside	+	+
8	Amino acids	+	+
9	Steroids	+	+
10	Phenols	+	+

"+" Indicates Presence" "_" Indicates Absence"

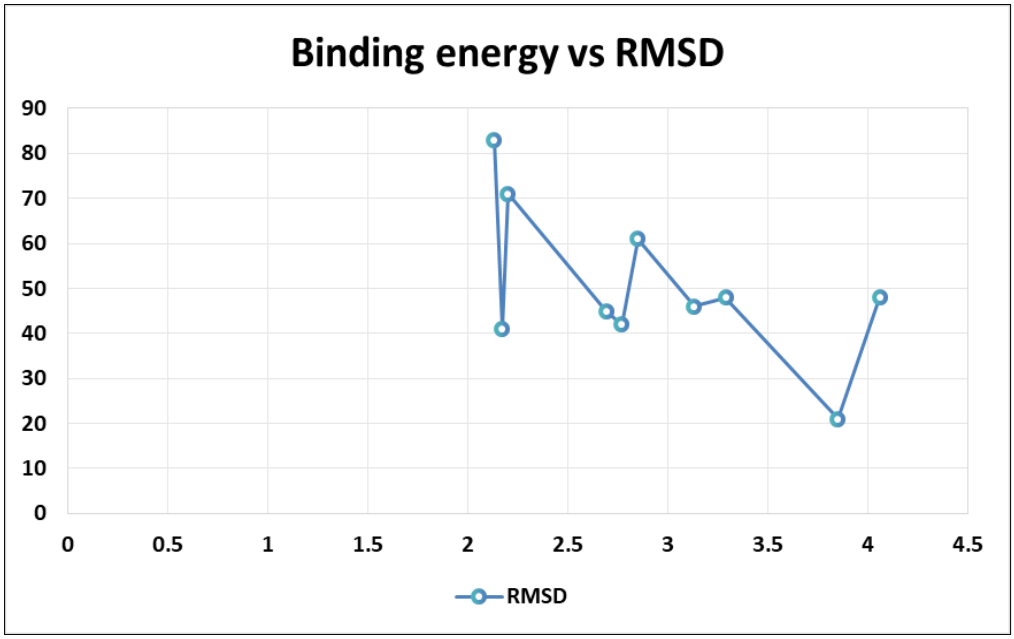

Fig 4: Quercetin Binding Capacity for Anti-Inflammatory Activity

Result

There is 72% binding capacity for Anti Inflammatory activity on Human Serum Albumin (HSA)

Table 6: RMSD- Binding Capacity of Anti-Inflammatory Activity

Sl.No	Sub Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	7	-4.06	0.00	48.65	RANKING
2	1	2	-3.85	0.00	21.51	RANKING
3	1	3	-3.29	0.00	48.84	RANKING
4	1	1	-3.13	0.00	46.08	RANKING
5	1	5	-2.85	0.00	61.83	RANKING
6	1	6	-2.77	0.00	42.03	RANKING
7	1	9	-2.69	0.00	45.74	RANKING
8	1	8	-2.20	0.00	71.28	RANKING
9	1	10	-2.17	0.00	41.85	RANKING
10	1	4	-2.13	0.00	83.23	RANKING

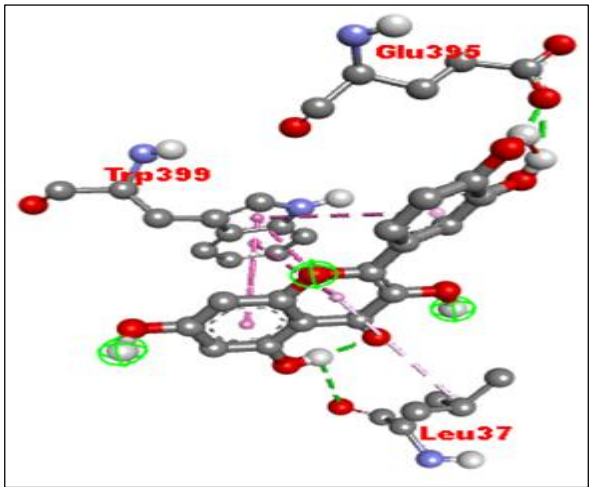

Fig 5: 3D Document of Quercetin Binding Capacity for Anti-Inflammatory Activity

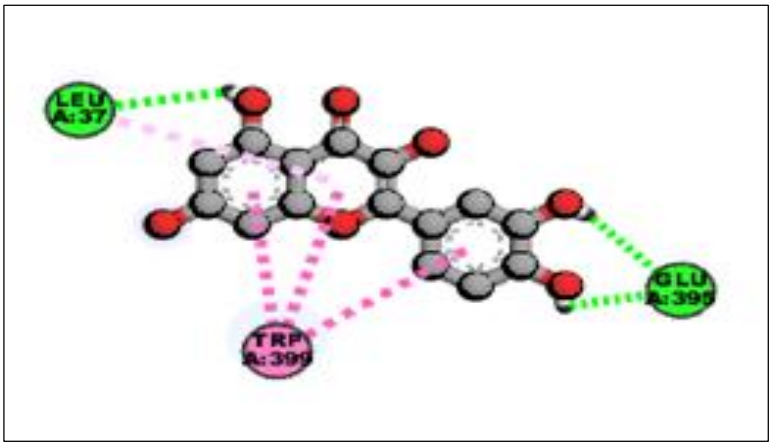

Fig 6: 2D Document of Quercetin Binding Capacity for Anti-Inflammatory Activity

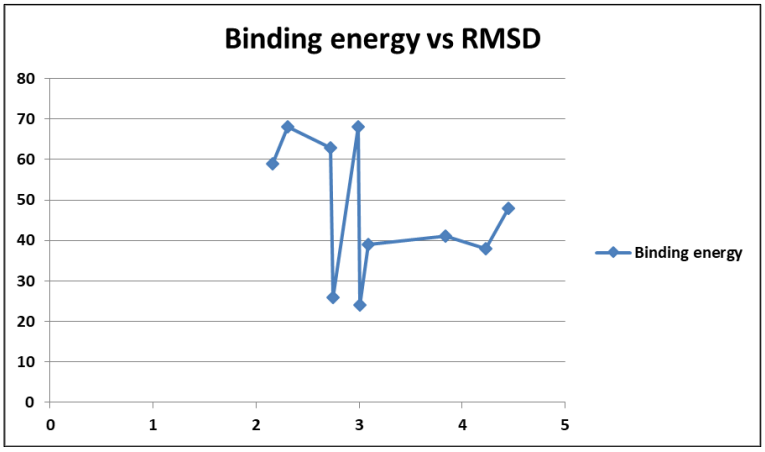

Fig 7: Quercetin Binding Capacity for Anti-Oxidant Activity

Result: There is 74% binding capacity for Anti-Oxidant activity on Human Serum Albumin (HSA)

Table 7: RMSD Binding Capacity of Anti-Oxidant Activity

Sl. No	Sub Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	2	-4.45	0.00	48.10	RANKING
2	1	6	-4.23	0.00	38.13	RANKING
3	1	3	-3.84	0.00	41.48	RANKING
4	1	1	-3.09	0.00	39.70	RANKING
5	1	8	-3.01	0.00	24.53	RANKING
6	1	5	-2.96	0.00	68.11	RANKING
7	1	9	-2.75	0.00	26.22	RANKING
8	1	4	-2.72	0.00	63.46	RANKING
9	1	10	-2.31	0.00	68.44	RANKING
10	1	7	-2.16	0.00	59.80	RANKING

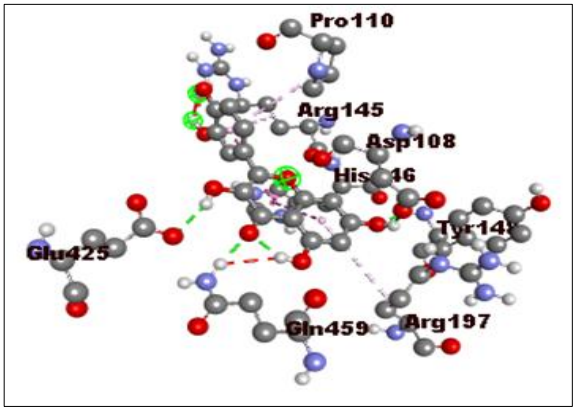

Fig 8: 3D Document of Quercetin Binding Capacity for Anti-Oxidant Activity

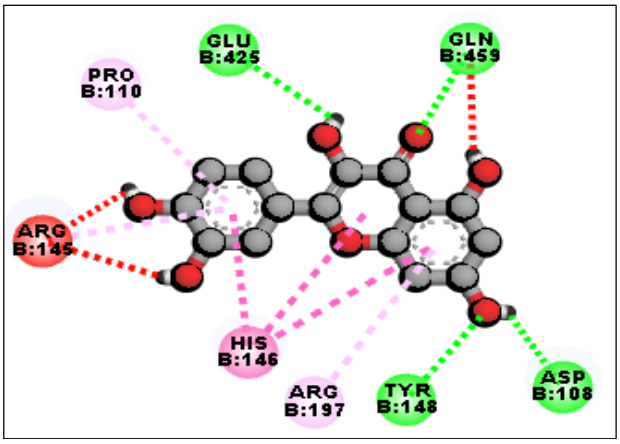

Fig 9: 2D Document of Quercetin Binding Capacity for Anti-Oxidant Activity

Table8: Hptlc Results of Peepal Tree Seeds on The Presence of Quercetin

Compound	Rf	Regression Equation	R2
Quercetin	0.07	Y= 6.9601 x + 5.18	0.9942

Rf and Linear regression equation of Quercetin

13. Discussion

The first study of its kind, this preliminary in silico study not only supports the potential of Peepal tree seeds as a therapeutic agent, as claimed by Ayurveda, but also lends scientific support to their therapeutic targets as potential candidates for healthcare in modern times.

However, much experimental validation and clinical studies are required to investigate the blood purification potential of Peepal tree seeds, supported by computational analysis.

14. Summary and Conclusion

This research focused on the extraction, phytochemical screening, and in silico evaluation of bioactive compounds from the seeds of the *Ficus religiosa* (Peepal tree).

Ethanolic extraction was carried out to isolate the phytoconstituents, followed by preliminary phytochemical analysis which confirmed the presence of flavonoids, phenolics, tannins, and alkaloids-compounds known for their therapeutic effects.

In silico molecular docking studies were employed to investigate the interaction of these bioactive compounds with key target proteins associated with oxidative stress and inflammation, such as COX-2, TNF-α, and IL-6 while Kidney and Lung diseases such as Cirrhosis and Nephrosis etc when the Blood purification process is not on point due to these diseases. Quercetin the well-known Chemical constituent stimulates the working for Blood purification by Immbolising the ROS (Reactive Oxygen Species) through anti-oxidant activity and removal of the harmful species through urine by anti-inflammatory activity.

The presence of key bioactive compounds with strong in silico binding affinities towards inflammatory and oxidative stress-related proteins suggests that these seeds may contribute to blood purification and overall systemic detoxification.

An HPTLC method has been developed with some modifications and it can be used for the simultaneous quantitative determination of quercetin in Peepal tree seeds; its main advantages are its simplicity, accuracy, and selectivity. This method can also be used for the estimation of these compounds in other herbal preparations and may be useful for standardization purposes.

15. References

1. Rozylo JK, Janicka M. Different planar techniques for prediction of solute retention in column liquid chromatography. J Planar Chromatogr. 1996;9(6):418-424.
2. Swaroop A, Gupta P, Sinha AK. Simultaneous determination of quercetin, rutin and coumaric acid in flowers of *Rhododendron arboreum* by HPTLC. *Chromatographia*. 2005;62(11-12):649-652.
3. Prasad PV, Subhakthe PK, Narayana A, Rao MM. Medico historical study of "asvattha" (sacred fig tree). Bull Indian Inst Hist Med Hyderabad. 2006;36:1-20.
4. Boots AW, Haenen GR, Bast A. Health effects of quercetin: from antioxidant to nutraceutical. Eur J Pharmacol. 2008;585(2-3):325-337.
5. Boots AW, Wilms LC, Swennen ELR, Kleinjans JCS, Bast A, Haenen GRMM. *In vitro* and ex vivo anti-inflammatory activity of quercetin in healthy volunteers. Nutrition. 2008;24(7-8):703-710.
6. Hirpara KV, Aggarwal P, Mukherjee J, Joshi N, Burman AC. Quercetin and its derivatives: Synthesis, pharmacological uses with special emphasis on anti-tumor properties and prodrug with enhanced bio-availability. Anticancer Agents Med Chem. 2009;9:138-161.
7. Chandrasekar SB, Bhanumathy M, Pawar AT, Somasundaram T. Phytopharmacology of *Ficus religiosa*. *Pharmacogn Rev*. 2010;4(8):195-199.
8. Biswas D. *In vitro* anthelmintic activity of *Ficus benghalensis*, *Ficus carica* & *Ficus religiosa*: a comparative anthelmintic activity. *Int J PharmTech Res*. 2011;3:152-153.
9. Gupta S, Gupta R. Detection and quantification of quercetin in roots, leaves and flowers of *Clerodendrum infortunatum* L. Asian Pac J Trop Dis. 2012;2(Suppl):S940-S943.
10. Singh S, Jaiswal S. Therapeutic properties of *Ficus religiosa*. *Int J Eng Res Gen Sci*. 2014;2(5):2091-2730.
11. Wang TY, Li Q, Bi KS. Bioactive flavonoids in medicinal plants: Structure, activity and biological fate. *Asian J Pharm Sci*. 2018;13:12-23.
12. Manzoor MF, Hussain A, Sameen A. Novel extraction, rapid assessment and bioavailability improvement of quercetin: A review. *Ultrason Sonochem*. 2021;78:105686.
13. Bastin A, Teimouri M. *In vitro* and molecular docking analysis of quercetin as an anti-inflammatory and antioxidant. *Curr Pharm Des*. 2023;29(11):883-891.