INFORMATICS JOURNALS

Research Article

Journal of Natural Remedies
DOI: 10.18311/jnr/2026/55918

eISSN: 2320-3358 | Vol 26(9), 2026 | pp. 2453-2469

Antioxidant and Hepatoprotective Potentials of *Bauhinia blakeana* Methanolic Leaf Extract: *In Vitro* and *In Vivo* Evaluation

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Abstract

Background: Oxidative stress is a key contributor to the onset and progression of hepatic disorders, where excessive free radicals impair cellular structures. Natural antioxidants are known to mitigate such damage and support liver function. *Bauhinia blakeana*, widely utilised in traditional medicine, has not yet been scientifically validated for antioxidant as well as hepatoprotective potential. **Aim:** This research aimed to investigate the antioxidant as well as hepatoprotective efficacy of the Methanolic Leaf Extract of *Bauhinia blakeana* (BbME) through both *in vitro* and *in vivo* experimental models. **Methods:** Phytochemical Profiling was conducted to identify major bioactive constituents. Antioxidant activity was quantified using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and hydrogen peroxide scavenging assays, with ascorbic acid as a standard. Hepatoprotective activity was evaluated in rats subjected to paracetamol-induced hepatotoxicity by analysing biochemical markers, viz., Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline phosphatase (ALP), and total bilirubin. Histopathological assessments were also performed to evaluate hepatic tissue integrity. **Results:** The extract contained flavonoids, tannins, triterpenes, and saponins. BbME exhibited strong free radical scavenging capacity, showing IC_{50} values comparable to those of ascorbic acid. *In vivo* studies demonstrated substantial hepatoprotection, reflected by significant normalisation of liver enzyme levels and restoration of hepatic architecture. The effects of BbME at higher doses were comparable to those of silymarin, a reference hepatoprotective agent. **Conclusion:** BbME demonstrates potent antioxidant and hepatoprotective properties, supporting its ethnomedicinal relevance. Further isolation of its active compounds and mechanistic exploration could establish its potential as a plant-based hepatoprotective therapeutic.

Major Findings: The extract of *B. blakeana* shows good hepatoprotective acidity. The docking score supports the idea that the presence of flavonoids, tannins, triterpenes, and saponins is responsible for it.

Keywords: Antioxidant Activity, *Bauhinia blakeana*, Hepatoprotective Activity, Molecular Docking, Paracetamol-induced Hepatotoxicity

Abbreviations: ABTS – 2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid); ALP – Alkaline Phosphate; ALT – Alanine aminotransferase; AST – Aspartate Aminotransferase; BbME - *Bauhinia blakeana* Methanolic Extract; DPPH - 2,2-diphenyl-1-picrylhydrazyl; H_2O_2 – Hydrogen Peroxide; NAPQI – N-acetyl-p-benzoquinone; PCM – Paracetamol; ROS – Reactive Oxygen Species; TB – Total bilirubin.

(Article Chronicle: Article Received on: 21.04.2026; Revised on: 17.07.2026; Accepted on: 15.09.2026)

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1. Introduction

Herbal medicine has been an important part of healthcare for thousands of years, serving as a primary therapeutic approach in nations such as India, Egypt, China, and Greece. Traditional medical frameworks, like *Ayurveda*, *Unani*, and Chinese medicine, have emphasised the therapeutic importance of plants and their phytoconstituents for disease prevention and treatment[1,2]. In recent decades, the global reliance on herbal formulations has increased, driven by concerns regarding the side effects of synthetic drugs and the search for holistic approaches to health management[3]. According to the World Health Organisation (WHO), a substantial portion of the global population relies on herbal remedies for primary medical needs, particularly in developing regions[4]. This revival of interest underscores the importance of validating traditional knowledge through scientific research and exploring the medicinal plant's pharmacological potential in the prevention and management of complex diseases, such as liver disorders.

Liver, an important organ with various physiological functions, including metabolism, detoxification of xenobiotics, storage of glycogen and vitamins, and production of bile acids required for digestion[5]. Hepatocytes, the most important cell type of the liver, work in concert with Kupffer cells, endothelial cells, and biliary canaliculi to maintain homeostasis[6]. However, this organ's central role in metabolism makes it vulnerable to injury from alcohol, toxins, drugs, and infections[7,8]. Despite its remarkable regenerative capacity, prolonged exposure to toxic insults may overwhelm its protective mechanisms, leading to acute or chronic liver injury. Paracetamol (acetaminophen)-induced hepatotoxicity is a well-known model of drug-mediated hepatic injury and a prevalent manifestation of acute liver failure globally[9,10]. Under normal conditions, paracetamol is metabolised safely; however, in overdose situations, the cytochrome P450 enzyme system generates the reactive intermediate *N*-acetyl-*p*-benzoquinone imine (NAPQI). On conjugation with glutathione, NAPQI is formed, but during overdose, hepatic glutathione stores are depleted, resulting in NAPQI buildup[11]. This activates oxidative stress, mitochondrial dysfunction, hepatocyte necrosis, and subsequent acute liver injury[12]. The clinical burden of drug-induced hepatotoxicity highlights the urgent need for hepatoprotective agents capable of preventing oxidative liver damage. In hepatic injuries, the key factor that plays a role in the onset and progression of injuries is Oxidative stress. Injury emerges when Reactive Oxygen Species (ROS) are produced and the antioxidant defence system is in balance[13]. ROS are normal metabolic by-products, but their overproduction results in damage to lipids, proteins, and DNA, eventually predisposing them to hepatocellular carcinoma[14]. Given the central role of oxidative stress in liver injury and systemic diseases, antioxidants have attracted significant attention as protective agents. Antioxidants neutralise ROS by donating electrons, thereby stabilising reactive molecules and preventing cellular damage. Endogenous antioxidant systems act as the body's first line of defence[15]. These enzymes detoxify ROS and maintain redox balance at the cellular level. Uric acid, another endogenous antioxidant, acts as a potent scavenger of ROS in the bloodstream. However, endogenous systems may become insufficient under conditions of excessive oxidative stress, necessitating external supplementation[16]. *Bauhinia blakeana* (Hong Kong orchid tree), an ornamental plant native to Hong Kong and Southeast Asia[17]. Traditionally used in herbal medicine for treating dysentery, diarrhoea, and skin disorders, the plant has been shown to contain phytochemicals, which are flavonoids, tannins, terpenoids, and phenolic compounds with strong antioxidant activity, found on phytochemical assessments. Importantly, its flavonoid-rich extracts may enhance antioxidant enzyme activity, suppress pro-inflammatory pathways, and activate protective transcription factors such as Nrf2, thereby providing hepatoprotection[18]. Pharmacokinetic studies on Bauhinia-derived flavonoids reveal efficient intestinal absorption, hepatic phase II metabolism (glucuronidation and sulfation), and distribution to liver tissues, followed by renal and faecal excretion. Such pharmacokinetic behaviour supports their biological relevance in protecting hepatocytes against oxidative damage. Despite these indications, their antioxidant and hepatoprotective activities have not been experimentally validated.

Therefore, this study was designed to systematically assess the *in vitro* antioxidant potential and the *in vivo* hepatoprotective potential against paracetamol-induced

hepatotoxicity. By explaining the antioxidant and hepatoprotective mechanisms of *B. blakeana*, this research seeks to provide a scientific rationale for its traditional use, establish its pharmacological profile, and contribute to the development of novel plant-derived hepatoprotective agents.

2. Materials and Methods

2.1 Materials

Analytical-grade chemicals and solvents (Loba Chemie, Labogen, India) were used, including methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Hydrogen Peroxide (H_2O_2), and Paracetamol (PCM). Silymarin (Silybon-140) was purchased from Micro Labs Ltd, India. Instruments included an Ultraviolet-Visible Spectroscopy (UV-Vis) spectrophotometer, biochemical analyser, rotary evaporator, balances, homogeniser, centrifuges, hot air oven, and Soxhlet apparatus.

2.2 Collection and Authentication of Plant Material

Fresh leaves of *B. blakeana* Dunn were collected in November 2023 from St. John Technical and Educational Campus, Palghar, Maharashtra. The specimen was taxonomically identified and authenticated by Dr R. K. Choudhary, Scientist, Agharkar Research Institute, Pune (Authentication No. AUTH 24-64; dated 28/11/2023).

2.3 Preparation of Extract

Leaves of *B. blakeana* were shade-dried, coarsely powdered, and sieved (#40 mesh). About 200g of powdered material was subjected to sequential Soxhlet extraction first with petroleum ether to remove lipophilic impurities, followed by methanol for exhaustive extraction of active phytoconstituents. The methanolic fraction was filtered, concentrated under reduced pressure using a rotary evaporator, and dried in a desiccator. % extract yield was calculated relative to the dry weight of the starting material.

2.4 Preliminary Phytochemical Screening

Standard qualitative tests were performed to identify major phytochemical groups. Alkaloids were detected using Wagner's, Hager's, and Dragendorff's reagents; flavonoids by Shinoda and lead acetate tests; tannins by ferric chloride; triterpenes by Salkowski's test; and saponins by the foam test[19–21].

2.5 *In Vitro* Antioxidant Potential

Antioxidant potential of the methanolic extract was assessed by virtue of DPPH, ABTS, and H_2O_2 radical scavenging assays[22,23]. For the DPPH assay, varying amounts of BbME (10–50 µg/mL) were mixed with DPPH solution, and absorbance was recorded at 517nm after 30min[24]. In the ABTS assay, ABTS$^{\bullet+}$ was generated by mixing ABTS with potassium persulfate and incubating overnight; the scavenging activity of extracts was measured at 745nm. H_2O_2 scavenging was determined at 230nm using phosphate buffer. Ascorbic acid served as the assay standard. Radical scavenging activity (%) was calculated, and IC_{50} values were determined from concentration-response curves[24].

2.6 Experimental Animals

Male Wistar rats with a body weight of 150–200g were obtained from a registered breeder (Lasmi Biopharms Pvt. Ltd.; Reg. No. 1277/PO/RCDT/S/09/CCSEA). Animals were housed under standard laboratory conditions (23±2 °C with relative humidity at 50–60%, 12 h light/dark cycle) with free access to diet and water[25]. The Institutional Animal Ethics Committee gave its approval to each experiment (SJIPR/IAEC/05/23-24).

2.7 Acute Toxicity Study

A single oral dose of extract was administered to non-pregnant female rats via oral gavage. Following administration, food was withheld for 2 hours but not water. The animals were carefully observed during the first 4 hours post-dosing, intermittently over the first 24 hours, and subsequently once daily for 14 days to detect any indications of toxicity or death[26–29].

2.8 Dose Selection and Assessment of Hepatoprotective Activity

Acute toxicity assessment led to the selection of two safe dose levels (200 mg/kg and 400 mg/kg body weight). Activity was assessed in the PCM-induced hepatotoxicity model in Wistar rats. After 14 days of acclimatisation, all animals were randomised into five groups (n = 5). Group I served as the standard control

and received distilled water for 8 days. Group II received vehicle for 7 days and a single oral dose of PCM (3 g/kg b.w.) on day 8 to induce hepatotoxicity. Group III was given PCM (3 g/kg b.w.) on day 8 after receiving Silymarin (100 mg/kg/day) for 7 days. Groups IV and V received BbME pretreatment for 7 days at 200 mg/kg and 400 mg/kg/day, respectively, and PCM (3 g/kg b.w.) on day 8[30,31].

2.9 Biochemical and Histopathological Evaluation

Retro-orbital puncture was employed for collecting blood samples, and serum was analysed for liver biomarkers, which include ALT, AST, ALP, and Total Bilirubin (TB), using an auto-analyser[32]. Livers were removed and weighed, and finally preserved in 10% formalin for histopathological examination. Sections of paraffin embedded in 5µm, and staining was done with haematoxylin and eosin; structural changes under the light microscope were inspected[33].

2.10 Molecular Docking

The molecular docking analysis was done using the PyRx software (version 0.8). The crystal protein structure was obtained from the Protein Data Bank (PDB), and the proteins were downloaded with PDB codes: 1VKX, 3DEH, 3E6I, 5IKQ, and 6TYP, which were used for hepatoprotective activity. Water molecules and heteroatoms were removed, while hydrogen atoms were added, and rotatable bonds were optimised when applicable, resulting in a cleaned protein for docking. As per the available active site grid coordinates from the co-crystalline ligand-based is used in PyRx to place a grid around the coordinates identified for efficient docking. The ligand structures used were made available in SDF format, and further, using PyRx software, their energy was minimised using the Universal Force Field (UFF) algorithm to ensure the structures' stable conformations while allowing the rotatable bonds flexibility. The integrated AutoDock Vina engine in PyRx software docking was performed, with the cleaned receptor structures kept rigid, and ligands were allowed to be flexible to make interactions. Among the multiple binding energy scores (kcal/mol), the lowest-ranked binding mode was selected, which was generated among multiple poses for a single ligand. Docking results, such as binding strengths and how ligands fit into the protein, were examined and shown using PyRx. Then, they were checked again with Discovery Studio Visualiser 2019 to see how the ligands interact with specific amino acid residues in the active site. To make sure the docking method worked well, the ligands that were already known to bind to the proteins were docked again into their usual binding spots. The difference between where the computer predicted the ligands would be and where they actually are in the crystal structure was measured using Root Mean Square Deviation (RMSD). If the RMSD was less than 2.0Å, it showed that the docking process was reliable and gave consistent results.

2.11 Statistical Analysis

The mean ± SEM (standard error of the mean) was used to express all data. One-way Analysis of Variance (ANOVA) and Dunnett's post hoc test were used for statistical evaluation. (GraphPad Prism v5.0). At $p < 0.05$, significant differences were observed statistically[34].

3. Results and Discussion

3.1 Preliminary Phytochemical Assessment

BbME revealed the presence of flavonoids, tannins, saponins, and triterpenes, while alkaloids were absent. These constituents are well recognised for their antioxidant and hepatoprotective effects, suggesting that the biological activities of BbME may be attributed to these bioactive metabolites.

3.2 Acute Toxicity Study

The oral acute toxicity assessment indicated that BbME was safe at a dose of 2000 mg/kg body weight, as no mortality or behavioural abnormalities were noted throughout the 14-day observation period. Animals remained active and healthy, and there was no significant change in either body weight or food intake. Hence, 1/10th and 1/5th of this dose (200 and 400 mg/kg) were chosen for subsequent pharmacological evaluation, confirming the extract's

non-toxic nature as per the Organisation for Economic Co-operation and Development (OECD) guidelines.

3.3 *In Vitro* Antioxidant Potential

3.3.1 DPPH-Induced Radical Scavenging Assay

The antioxidant potential of BbME was assessed employing the DPPH radical scavenging assay. At all doses (10–50 µg/mL), a dose-dependent rise in free radical scavenging potential was noted, similar to that of ascorbic acid (Figure 1a). The IC_{50} value of BbME was 29.72 µg/mL, whereas ascorbic acid showed an IC_{50} of 25.44 µg/mL (Figure 1b). Although the extract demonstrated slightly lower activity than the standard, its relatively close IC_{50} value indicates significant free radical scavenging potential.

3.3.2 ABTS-induced Radical Scavenging Assay

This study revealed that BbME demonstrated notable antioxidant activity in a concentration-dependent manner, comparable to standard Trolox (Figure 2). At the highest tested concentration (50 µg/mL), BbME exhibited 75.15% scavenging activity, while Trolox showed 66.87%. IC_{50} value of BbME was determined to be 30.98 µg/mL, slightly higher than that of Trolox (28.59 µg/mL), indicating that although the extract possessed slightly lower potency than the standard, it still displayed significant radical scavenging potential.

3.3.3 H_2O_2 (Hydrogen Peroxide)- induced Radical Scavenging Assay

The study demonstrated a clear dose-dependent response at 50–300 µg/mL (Figure 3). % inhibition observed for the extract was 32.06%, 43.05%, 52.36%, 65.57%, 79.54%, and 87.32%. A comparable dose-dependent scavenging effect was also noted for the standard antioxidant, ascorbic acid. The IC_{50} value (131 µg/mL) was slightly higher than that of ascorbic acid (112 µg/mL), suggesting that BbME shows moderately lower potency but retains significant ROS-neutralising capability (Figure 3).

3.4 Effect of BbME on Liver Function Markers

PCM at toxic doses undergoes hepatic metabolism to produce the reactive metabolite NAPQI, which depletes glutathione and initiates oxidative stress, leading to hepatocellular damage and leakage of marker enzymes. PCM administration (3 g/kg) demonstrated a rise in serum levels of ALT, AST, and ALP, as well as TB levels, compared with the control group ($p < 0.01$), confirming liver injury. Pretreatment with silymarin (100 mg/kg) significantly ($p < 0.01$) restored these biochemical parameters toward normal. Similarly, BbME at 400 mg/kg markedly reduced elevated enzyme levels ($p < 0.01$), while the 200 mg/kg dose offered only limited protection. These findings (Table 1) demonstrate that BbME exerts a dose-dependent

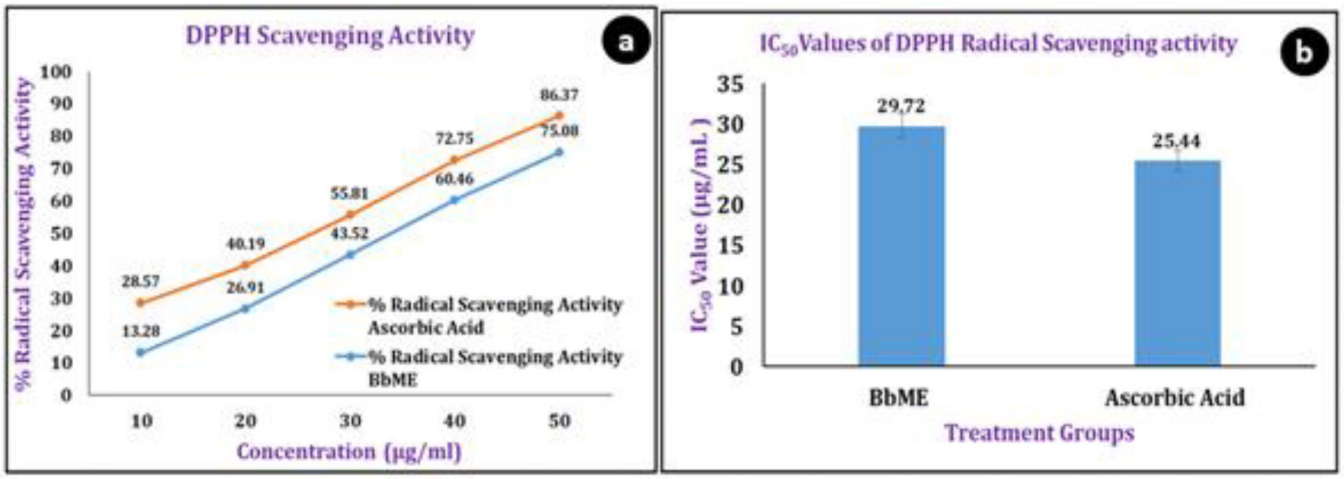


Figure 1. **(a).** DPPH-induced radical scavenging assay, **(b).** IC_{50} value of DPPH-induced radical scavenging assay.

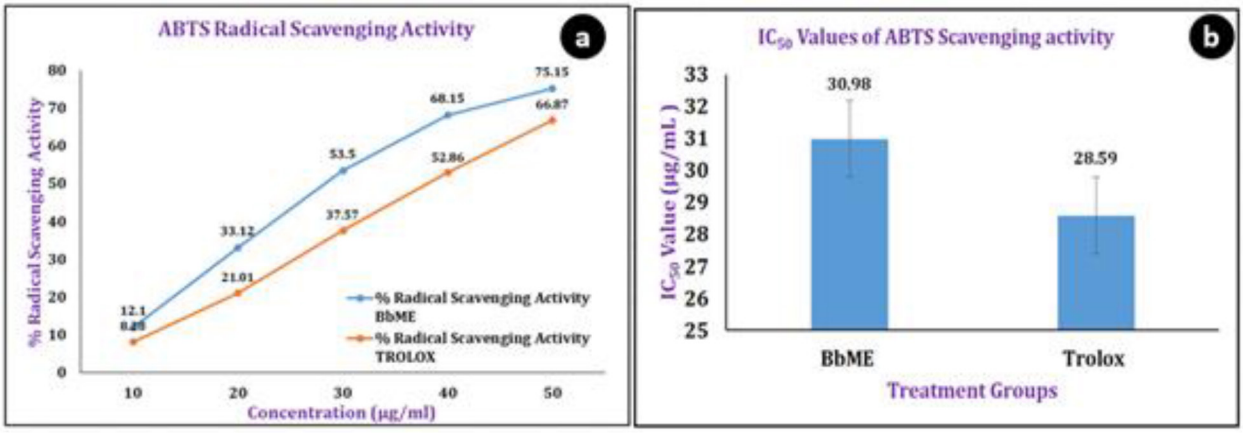


Figure 2. **(a).** ABTS-induced radical scavenging assay, **(b).** IC_{50} value for the ABTS-induced radical scavenging assay.

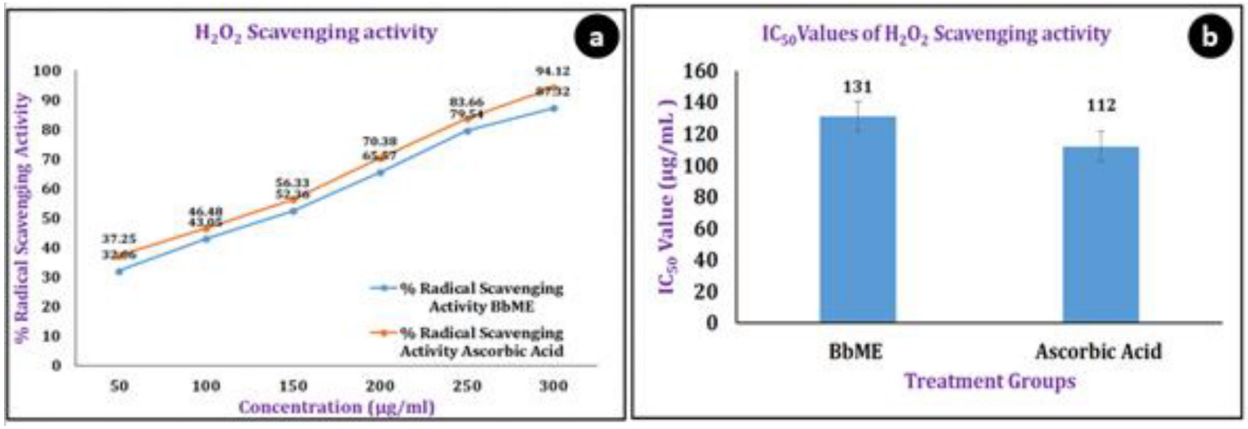


Figure 3. **(a).** H_2O_2-induced radical scavenging assay, **(b).** IC_{50} value for the H_2O_2-induced radical scavenging assay.

Table 1. Effects of BbME on serum ALT, AST, ALP, and TB levels in rats

Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)	TB (mg/dL)
Normal Control (G–I)	92.40 ± 1.50	94.80 ± 1.35	160.2 ± 1.46	0.154 ± 0.143
Disease Control PCM 3 g/kg (G–II)	660.2 ± 3.05#	548.0 ± 2.77#	678.4 ± 3.98#	0.666 ± 0.016#
Silymarin 100 mg/kg + PCM 3 g/kg (G–III)	145.4 ± 1.86**	115.8 ± 1.35**	207.8 ± 2.35**	0.220 ± 0.014**
BbME 200 mg/kg + PCM 3 g/kg (G–IV)	649.2 ± 1.11*	538.2 ± 1.24*	666.0 ± 2.21*	0.570 ± 0.018*
BbME 400 mg/kg + PCM 3 g/kg (G–V)	162.6 ± 2.73**	143.2 ± 2.15**	236.2 ± 1.39**	0.268 ± 0.015**

*Values are expressed as mean ± SEM (Standard error of the mean) of n=5 rats in each group. Significance level: #p < 0.01 compared to the control group (G-I), *p < 0.05, and *p < 0.01 compared to the disease control group (G-II).

hepatoprotective potential, comparable to silymarin, against PCM-induced hepatotoxicity.

3.5 Histopathological Study

Liver sections from the control group exhibited normal histoarchitecture, characterised by intact hepatocytes and a well-defined central vein. PCM-treated rats (Group II) exhibited severe hepatocellular necrosis, macrovesicular steatosis, congestion, leukocyte infiltration, and fatty degeneration. Silymarin treatment (Group III) markedly reduced necrotic changes with only mild

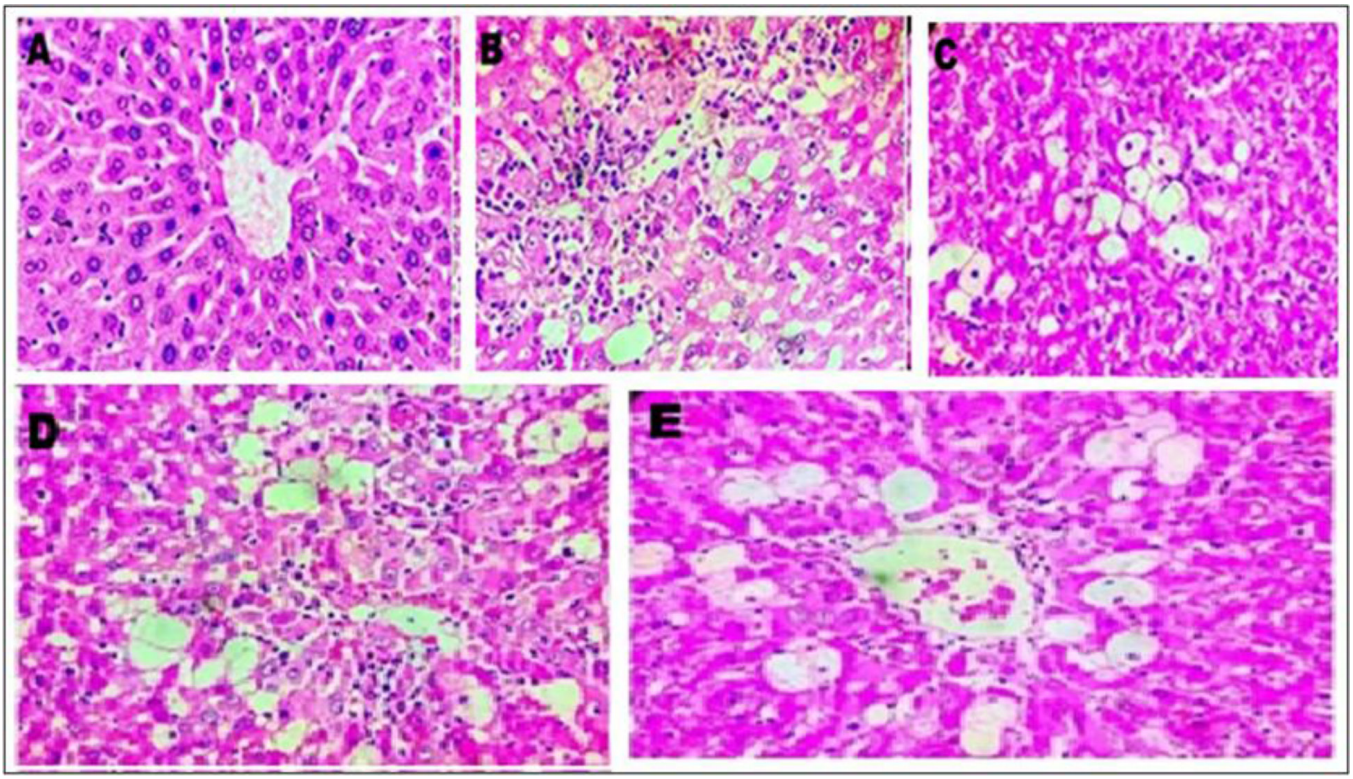


Figure 4. Histopathology of liver tissues of rats in the control group and the test groups at 40X magnification. **(a).** Normal control group, **(b).** Disease control group, **(c).** Standard drug-treated group III, **(d).** Extract-treated BbME 200 mg/kg group IV, and **(e).** Extract-treated BbME 400 mg/kg group V.

macrovesicular alterations. Rats treated with BbME at 200 mg/kg (Group IV) displayed moderate hepatocyte changes and mild inflammation, while BbME at 400 mg/kg (Group V) demonstrated near-normal architecture with minimal cellular damage, reduced inflammation, and improved hepatocyte regeneration. Overall, BbME, particularly at higher doses, significantly attenuated paracetamol-induced liver injury, reflecting hepatoprotective properties comparable to silymarin. (Figure 4) illustrates the results of a histopathological study.

3.6 Molecular Docking

The selected proteins and ligand structures were docked using PyRx, and their binding affinities were predicted. The binding affinities of Rutin and Quercetin-3-rhamnoside were higher than those of the other compounds, indicating their stronger interactions with the target. Rutin and Quercetin-3-rhamnoside interacted with 1VKX, 3DEH, 3E6I, 5IKQ, and 6TYP. Rutin and Quercetin-3-rhamnoside, as ligands, showed good binding affinities among all 5 proteins. Rutin has the highest binding affinity in 1VKX, 3E6I, 5IKQ, and 6TYP compared to other plant constituents (-10.3, -8.4, -10.1, and -9.7 kcal/mol), whereas in 3DEH, Quercetin has the highest binding affinity with (-9.2 kcal/mol). The 2D and 3D structures of Rutin and Quercetin-3-rhamnoside interact with plant constituents with several bonds (Figures 5a-5j). Thus, the combined molecular evidence strongly supports the hepatoprotective potential of the plant extract, indicating that all constituents together exhibit the activity. These findings justify further *in vitro* enzymatic inhibition studies and *in vivo* hepatoprotective assays to validate their therapeutic relevance (Table 2 and Figure 6).

Table 2. Binding affinities and protein interactions of selected phytochemicals

Phytochemical	Protein Binding Affinity IDs					Type of Bonding and Interaction
	1VKX	3DEH	3E6I	5IKQ	6TYP	
Apigenin	-8	-9	-7.8	-8.5	-8.8	Conventional H-bond – SER B:363 Pi-Sigma – VAL B:412, LEU B:440 Pi-Alkyl/Alkyl – ARG B:356, VAL B:358
						Conventional H-bond – ASN A:208, GLN A:217, GLU A:246, GLU A:248, PHE C:252 Carbon H-bond – PHE A:247 Pi-Pi T-shaped – TRP A:214, PHE C:252
						Conventional H-bond – TYR B:310, ASP B:394 Pi-Alkyl/Alkyl – PRO B:473 Pi-Pi Stacked – TYR B:398 Pi-donor H bond – TYR B:398
						Conventional H-bond – TRP B:324 Pi-Alkyl/Alkyl – CYS A:36, CYS A:47, PRO A:153, PRO A:156 Pi-donor H-bond – ASN A:34, GLN B:328
						Conventional H-bond – VAL A:512 Pi-Alkyl/Alkyl – ALA A:366, ALA A:415, ALA A:556
Isorhamnetin	-7.4	-7.9	-7.7	-9.3	-9.1	Conventional H-bond – SER B:363, GLY B:365, ILE B:439 Pi-Sigma – VAL B:412, LEU B:440 Pi-Alkyl/Alkyl – PRO B:362
						Conventional H-bond – THR A:166, HIS C:121 Pi-Alkyl/Alkyl – HIS C:121, TYR C:204 Carbon H-bond – THR A:255 Pi-Pi T-shaped – TYR C:204, PHE A:256
						Conventional H-bond – ASN B:52 Pi-Alkyl/Alkyl – PRO B:406 Pi-Pi T-shaped – TYR B:398 Pi-Anion – ASP B:394
						Conventional H-bond – ASN B:34, GLY B:45, GLY B:136 Pi-Sigma – PRO B:157 Pi-Alkyl/Alkyl – CYS B:36, CYS B:47, PRO B:154 Pi-Sulfur – CYS B:36
						Conventional H-bond – VAL A:418, VAL A:463, VAL A:465, THR A:560, VAL A:604 Pi-Alkyl/Alkyl – ALA A:366, CYS A:368, ALA A:607 Carbon H-bond – GLY A:417
Kaempferol	-7.6	-8	-7.8	-9.2	-9.1	Conventional H-bond – ARG B:356, GLY B:365, ASN B:436 Pi-Sigma – VAL B:412 Pi-Alkyl/Alkyl – PRO B:362, LEU B:440
						Conventional H-bond – ARG A:207, ASN A:208, TRP A:214, PHE A:250, SER C:251, ASP C:253 Pi-Sigma – PHE C:252 Pi-Pi T-shaped – PHE C:252
						Conventional H-bond – ASN A:52 Pi-Pi T-shaped – TYR A:398 Pi-Anion – ASP A:394
						Conventional H-bond – CYS B:36, HIS B:39, CYS B:47 Pi-Alkyl/Alkyl – VAL B:46, PRO B:154 Pi-donor H bond – CYS B:47, TYR B:131
						Conventional H-bond – ALA A:510 Pi-Alkyl/Alkyl – ALA A:366, ARG A:415, ALA A:556 Carbon H-bond – GLY A:464

Table 2. Continued...

Phytochemical	Protein Binding Affinity IDs					Type of Bonding and Interaction
	1VKX	3DEH	3E6I	5IKQ	6TYP	
Myricetin	-8.1	-8.2	-7.8	-8.9	-9.5	Conventional H-bond – HIS B:364, GLY B:365 Pi-Alkyl/Alkyl – ARG B:356, VAL B:412, LEU B:440
						Conventional H-bond – GLY A:122, ARG A:164 Pi-Pi T-shaped – TYR A:204, PHE C:256
						Conventional H-bond – ASN B:52, THR B:58, LEU B:471 Pi-Alkyl/Alkyl – PRO B:473 Pi-Pi T-shaped – TYR B:398 Pi-donor H bond – TYR B:398 Pi-Sulfur – CYS B:480
						Conventional H-bond – HIS A:39, GLY A:45, SER A:49, PRO A:154, GLN B: 158 Pi-Alkyl/Alkyl – CYS A:36, CYS A:47, PRO A:153, PRO A:156 Carbon H bond – ASN A:34, VAL A:46 Pi-Pi T-shaped – TYR A:136 Pi-donor H-bond – ASN A:34, VAL A:46
						Conventional H-bond – VAL A:463, VAL A:465, ALA A:5110, ILE A:559, VAL A:606
Quercetin-3-rhamnoside	-7	-9.2	-8.1	-9.1	-9.1	Conventional H-bond – ARG A:33, ALA A:43, SER A:45, SER A:51, THR A:57 Pi-Alkyl/Alkyl – MET A:32, PRO A:47 Carbon H-bond – GLY A:44
						Conventional H-bond – THR A:166, THR C:62, GLY C:165, THR C:165 Pi-Pi T-shaped – TYR C:204, PHE A:256
						Conventional H-bond – ASN A:52, ILE A:361 Pi-Alkyl/Alkyl – PRO B:473, ILE A:476 Pi-Pi T-shaped – PHE A:360 Pi-donor H bond – TYR B:398 Pi-Anion – ASP A:394 Pi-Sulfur – CYS A:480
						Conventional H-bond – SER B:49, ASP B:158 Pi-Alkyl/Alkyl – PRO B:155, PRO B:157 Carbon H bond – GLN A:327 Pi-Pi T-shaped – TYR B:137 Pi-donor H bond – GLN A:327 Pi-Anion – ASP B:158
						Conventional H-bond – LEU A:365, SER A:508, VAL A:604 Pi-Sigma – ALA A:556 Pi-Alkyl/Alkyl – ARG A:415, ALA A:556 Carbon H-bond – GLY A:364, GLY A:462, GLY A:509, GLY A:603

Table 2. Continued...

Phytochemical	Protein Binding Affinity IDs					Type of Bonding and Interaction
	1VKX	3DEH	3E6I	5IKQ	6TYP	
Quercetin	-7.6	-7.9	-7.8	-8.6	-9.2	Conventional H-bond – ARG B:356, SER B:363 Pi-Sigma – VAL B:412, LEU B:440 Pi-Alkyl/Alkyl – ARG B:356, VAL B:412
						Conventional H-bond – GLY A:165 Pi-Pi T-shaped – TYR A:204, PHE C:256
						Conventional H-bond – ASN B:52, THR B:58, TYR B:310, THR B:362 ASP B:394 Pi-Alkyl/Alkyl – PRO B:473 Pi-Pi T-shaped – TYR B:398 Pi-donor H bond – TYR B:398 Pi-Sulfur – CYS B:480
						Conventional H-bond – CYS B:47, SER B:49, ASP B:134, PRO B:155, ASP B:158, LYS B:460 Pi-Alkyl/Alkyl – CYS B:36, PRO B:157 Pi-donor H-bond – GLY B:136 Pi-Anion – ASP B:158
						Conventional H-bond – VAL A:463, VAL A:465, ALA A:510, LEU A:557
Rutin	-10.3	-8.6	-8.4	-10.1	-9.7	Conventional H-bond – ARG B:356, TYR B:357, VAL B:358, GLY B:361, SER B:363, HIS B:364, GLY B:413, CYS B:416 Pi-Sigma – VAL B:412 Pi-Alkyl/Alkyl – PRO B:362 Carbon H-bond – GLY B:365 Amide Pi-Stacked – GLY B:361
						Conventional H-bond – THR A:166, GLY C:122, ARG C:164, GLY C:165 Pi-Sigma – THR C:166 Pi-Pi T-shaped – TYR A:204
						Conventional H-bond – TYR A:58, PHE A:360, THR A:362, ASP A:394 TYR A:398 Pi-Alkyl/Alkyl – PHE A:360 Carbon H bond – PRO A:473 Pi-Pi T-shaped – TYR A:398
						Conventional H-bond – CYS A:36, SER A:49, ASP A:133, GLY A:135, PRO A:154, GLN B:328 Pi-Anion – ASP A:157
						Conventional H-bond – SER A:363, LEU A:365, APG A:380, SER A:508, LEU A:557, TYR AA:572, VAL A:604 Pi-Alkyl/Alkyl – ALA A:556 Pi-Pi Stacked – TYR A:334

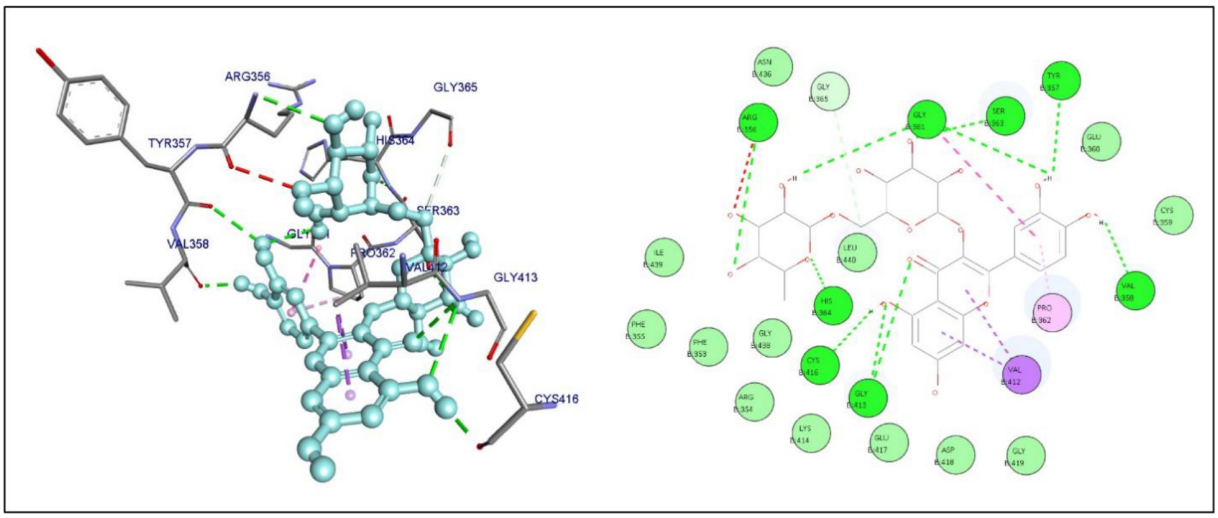


Figure 5a. Ligand Rutin and target 1VKX interaction.

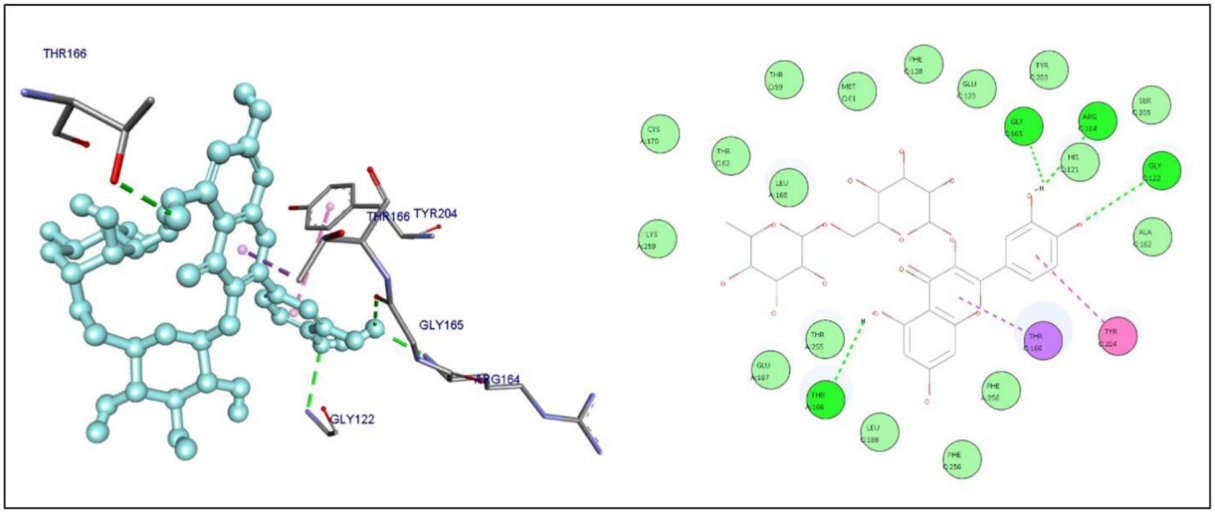


Figure 5b. Ligand Rutin and target 3DEH interaction.

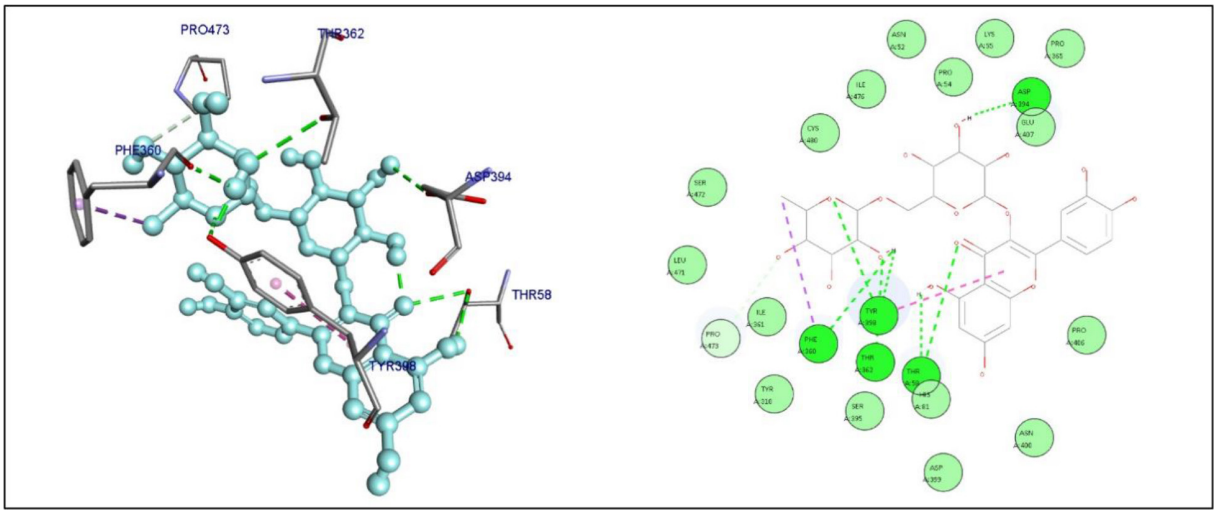


Figure 5c. Ligand Rutin and target 3E6I interaction.

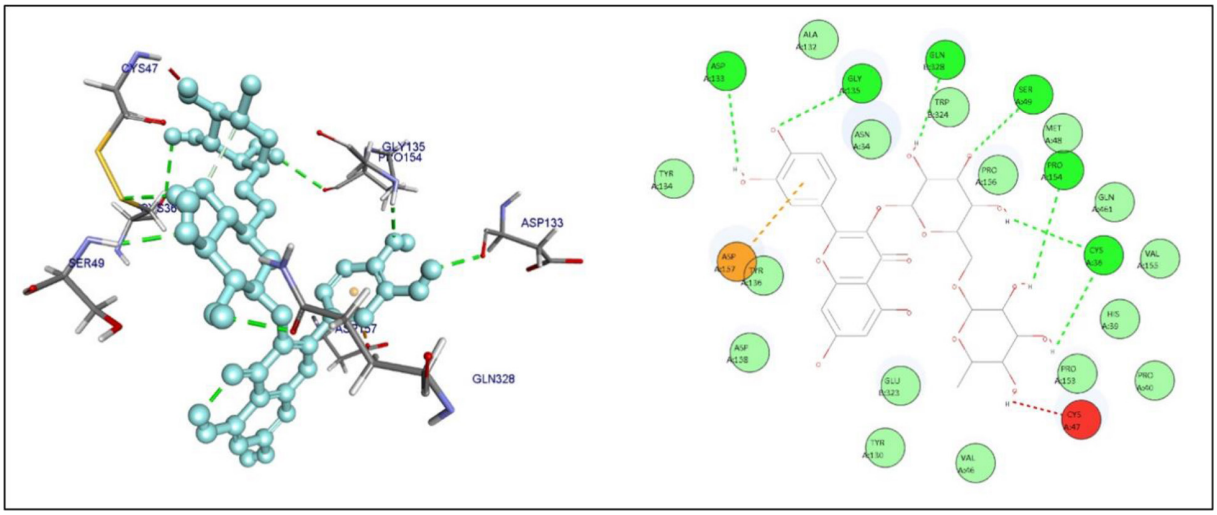


Figure 5d. Ligand Rutin and target 5IKQ interaction.

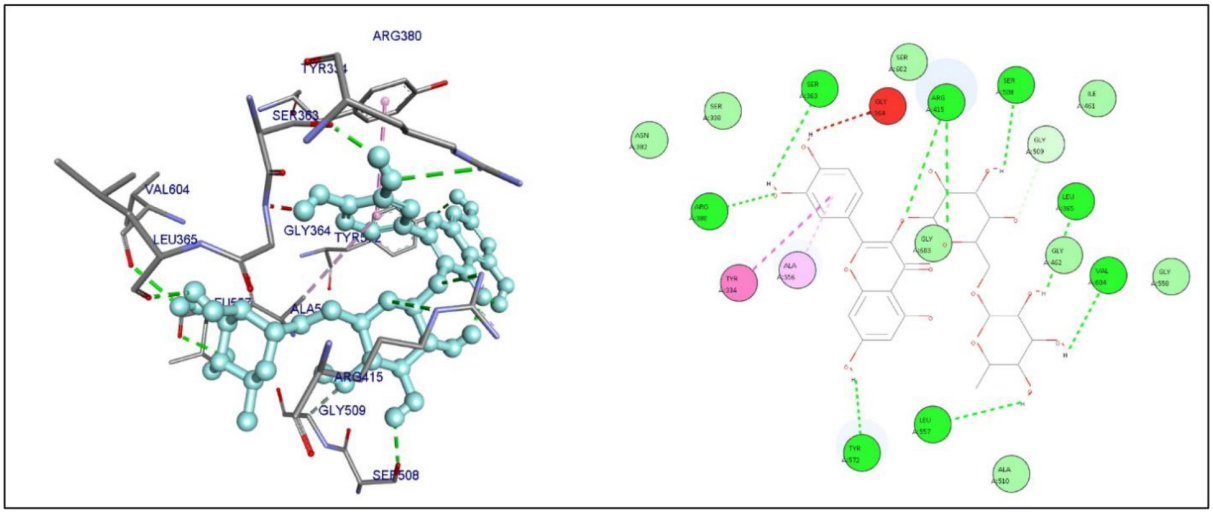


Figure 5e. Ligand Rutin and target 6TYP interaction.

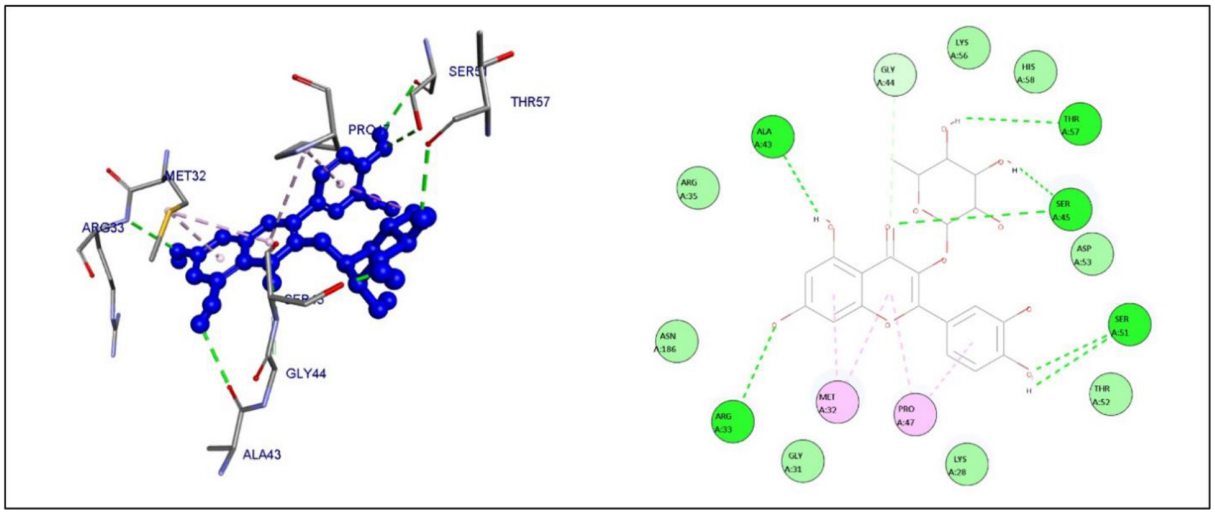


Figure 5f. Ligand Quercetin-3-rhamnoside and target 1VKX interaction.

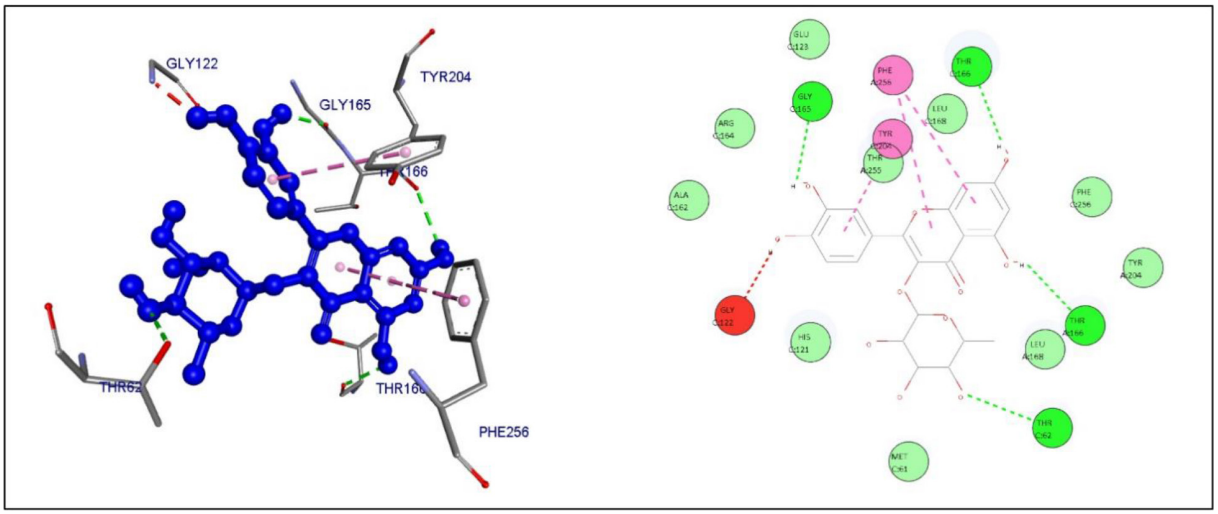


Figure 5g. Ligand Quercetin-3-rhamnoside and target 3DEH interaction.

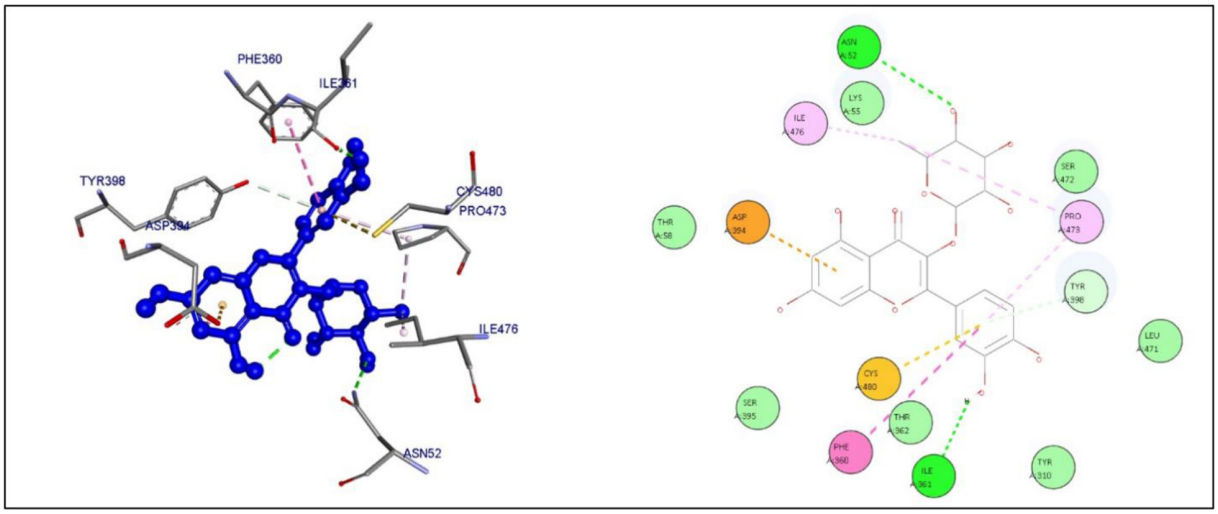


Figure 5h. Ligand Quercetin-3-rhamnoside and target 3E6I interaction.

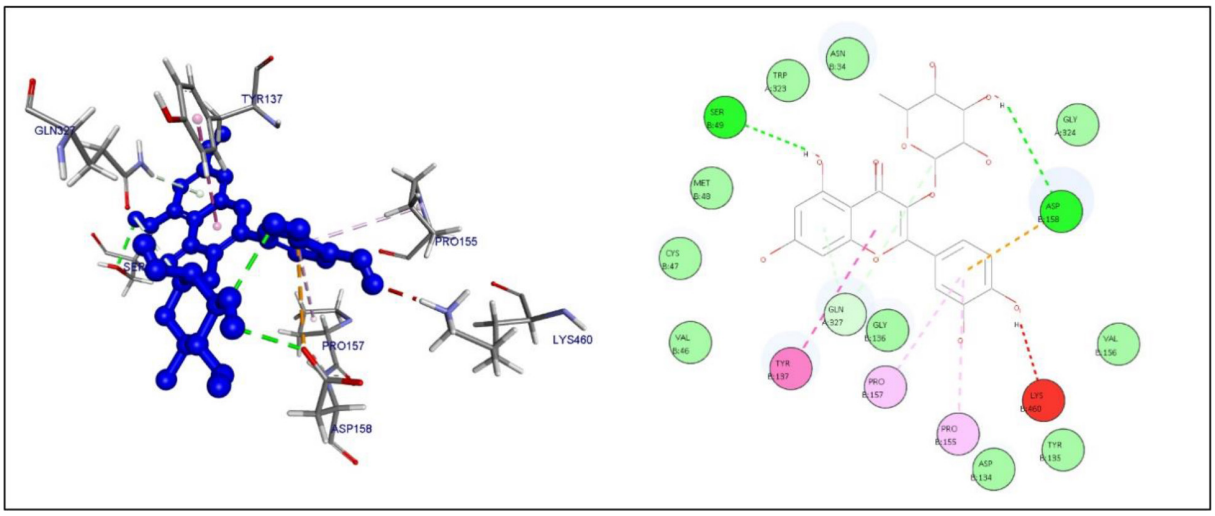


Figure 5i. Ligand Quercetin-3-rhamnoside and target 5IKQ interaction.

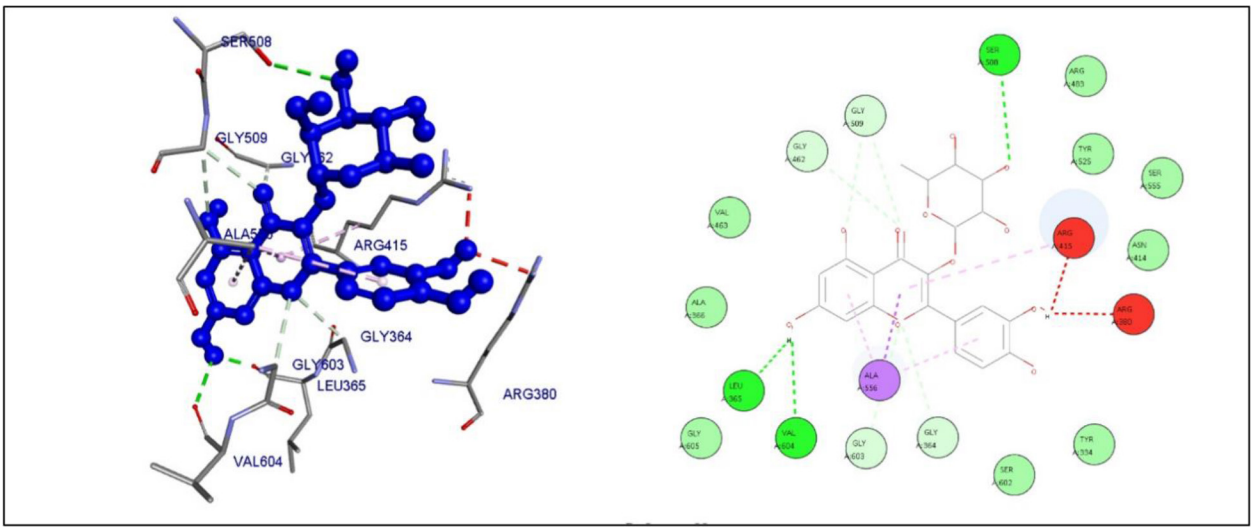


Figure 5j. Ligand Quercetin-3-rhamnoside and target 6TYP interaction.

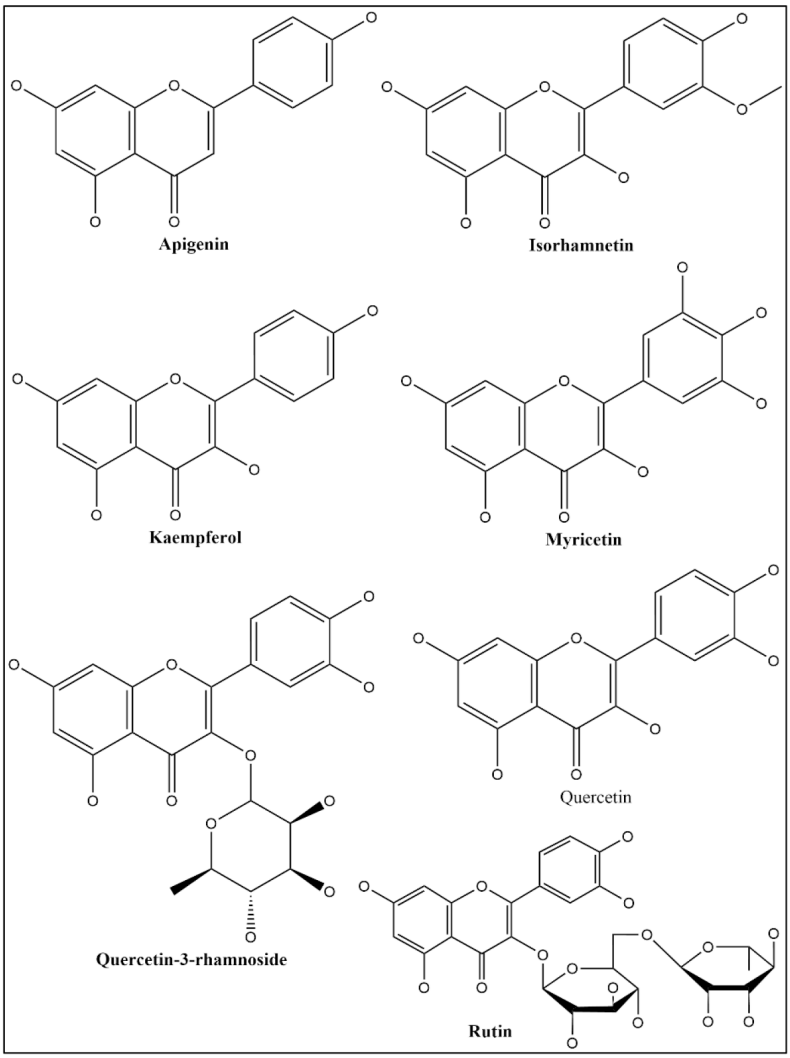


Figure 6. Chemical structures of major phytochemicals present in *Bauhinia blakeana*.

4. Conclusion

Findings of this study demonstrate that BbME possesses potent antioxidant and hepatoprotective activities, thereby providing strong pharmacological validation for its traditional use. Rich in flavonoids, tannins, saponins, and triterpenes, BbME showed robust free radical scavenging capacity and afforded significant protection against paracetamol-induced hepatotoxicity, with effects comparable to silymarin. The dual actions of direct antioxidant activity and preservation of hepatocellular integrity position BbME as a promising candidate for managing oxidative stress-mediated liver injury. These findings not only extend the therapeutic relevance of the *Bauhinia* genus but also underscore the potential of BbME as a lead for evidence-based phytotherapeutics. Future studies should focus on isolating active principles, elucidating molecular targets, and establishing clinical efficacy to advance its development.

5. Acknowledgement

The authors extend their highest appreciation to the Management at St. John Institute of Pharmacy and Research, Palghar, for providing the necessary research facilities to carry out the work.

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Author Contribution

D.B. and P.M: Conceptualisation, investigation. R.V. and D.P.: methodology, software, writing—original draft preparation. N.K. and P.M.: writing—original draft preparation, validation, formal analysis; S.B. and D.B.: writing—review and editing, project administration, visualisation; S.B., D.B., and P.M: supervision, data curation. All authors have read and agreed to the published version of the manuscript.