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ORIGINAL ARTICLE

In Silico Investigation of the Hepatoprotective Potential of *Hamelia patens* GC-FID Identified Compounds against Hepatotoxicity

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ABSTRACT

Hepatotoxicity remains a limitation in the management of chemical-induced liver injury, prompting interest in plant-derived compounds with multitarget hepatoprotective potential. This study investigated the phytochemical composition of *Hamelia patens* leaf extract and the in silico hepatoprotective potential of selected constituents against CYP2E1, COX-1 and NF-κB. *H. patens* leaves were authenticated, extracted with distilled water, and subjected to qualitative and quantitative phytochemical screening. Constituents were characterized and quantified by gas chromatography-flame ionization detection (GC-FID). Tangeretin, resveratrol and luteolin were selected from the identified compounds, while silymarin served as the reference ligand. Their drug-likeness, predicted toxicity and molecular interactions with CYP2E1, COX-1 and NF-κB were evaluated using Lipinski's Rule of Five, in silico toxicity prediction and AutoDock Vina, respectively. The extract contained abundant alkaloids (235.81 ± 1.47 mg/100 g), followed by flavonoids (146.68 ± 0.91), phenolics (95.89 ± 2.24), tannins (93.22 ± 2.01), glycosides (51.50 ± 1.88) and saponins (40.18 ± 1.06 mg/100 g). GC-FID identified 23 phytochemicals with a total concentration of 380.52 ppm; pinene (102.06 ppm), limonene (60.97 ppm) and tangeretin (42.42 ppm) predominated. All selected ligands satisfied Lipinski's criteria. Luteolin showed the strongest phytochemical binding to CYP2E1 (−9.1 kcal/mol; Ki = 0.21 µM) and COX-1 (−8.6 kcal/mol; Ki = 0.49 µM), while its NF-κB binding was −6.9 kcal/mol (Ki = 8.67 µM). Tangeretin, resveratrol and luteolin had predicted LD50 values of 5000, 1560 and 3919 mg/kg, respectively. *H. patens* contains diverse phytochemicals with favourable computational profiles and potential multitarget hepatoprotective effects. Further in vitro and in vivo studies are recommended to validate these predicted interactions and therapeutic potential.

Keywords: CYP2E1; *Hamelia patens*; Hepatoprotection; *In silico* toxicity; Luteolin; Molecular docking.

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1.0 INTRODUCTION

The liver, as the primary organ of xenobiotic metabolism, is inherently susceptible to chemical-induced injury, a growing global health concern with limited pharmacological interventions (Ramos-Tovar & Muriel, 2020). Carbon tetrachloride (CCl_4)-induced hepatotoxicity is widely used as an experimental model for investigating liver injury because its metabolic activation produces mechanisms of oxidative damage relevant to hepatotoxic processes (Khalid, 2022). CCl_4 is metabolized in the endoplasmic reticulum principally by cytochrome P450 2E1 (CYP2E1) to the highly reactive trichloromethyl radical ($CCl_3•$), which reacts with oxygen to form the trichloromethyl peroxyl radical ($CCl_3OO•$), initiating lipid peroxidation and subsequent hepatocellular injury (Hussein & Khan, 2022). CYP2E1 is therefore considered a major enzymatic mediator of CCl_4 bioactivation and a rational molecular target for investigating hepatoprotective interventions (Wong et al., 1998).

Beyond metabolic activation, inflammatory signalling contributes substantially to CCl_4-induced liver injury. Nuclear factor kappa B (NF-κB) has been identified as a central regulator of hepatic inflammation and cell death (Luedde & Schwabe, 2011), with Kupffer cell-mediated IKK/NF-κB signalling implicated in the inflammatory response to hepatic injury (He & Karin, 2011). Cyclooxygenases are involved in inflammatory processes that are linked to hepatic injuries, with experimental evidence suggesting that these can be mediated by the action of COX pathways in liver damage caused by CCl_4 and other hepatotoxic agents (Mohammed et al., 2004; Bahde et al., 2014). These mechanisms highlight CYP2E1, NF-κB, and COX-1 as molecular targets relevant to CCl_4-induced hepatotoxicity. COX-1 (rather than the inducible COX-2, which is more classically linked to inflammation) was selected here because of its constitutive, protective role in maintaining hepatic prostaglandin E2-mediated regeneration, making its interaction with candidate phytochemicals mechanistically informative even though, unlike CYP2E1, its inhibition is not per se a desirable outcome.

Hamelia patens Jacq. (Rubiaceae), commonly known as firebush, is a tropical medicinal plant that has been utilized in Mexico and Central America for a long time for medicinal purposes (Noor et al., 2020). Its leaves contain a variety of bioactive ingredients such as oxindole alkaloids, flavonoids, and phenolic compounds, including metabolites such as rutin, isoquercetin, and soyasaponin Bb reported by HPLC-ESI-MS (Gutiérrez-Sánchez et al., 2022); it should be noted that these specific compounds were not among those identified by GC-FID in the present study, which instead detected a distinct phytochemical profile dominated by terpenes and polymethoxy-flavones (Table 2). The HPLC-ESI-MS method has been employed to identify these compounds in *H. patens* leaves (Gutiérrez-Sánchez et al., 2022). Previous experimental evidence also confirms the hepatoprotective activity of *H. patens* extracts, with significant protective effects reported against CCl_4-induced damage in HepG2 cells (Perez-Meseguer et al., 2016). However, the molecular interactions through which individual *H. patens* phytochemicals may contribute to hepatoprotection remain insufficiently characterized.

In silico approaches, particularly molecular docking and ADMET profiling, provide useful tools for predicting interactions between bioactive compounds and disease-relevant molecular targets and for evaluating their

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drug-development potential (Nahar & Sarker, 2024; Kaushik et al., 2025). Molecular docking can provide insights into ligand-binding interactions and possible mechanisms through which plant-derived compounds may modulate target proteins (Nematallah et al., 2024). Despite the reported phytochemical diversity and hepatoprotective activity of *H. patens*, the interactions of its leaf phytochemicals with molecular targets associated with CCl_4-induced hepatotoxicity remain insufficiently investigated. Consequently, this research utilized drug-likeness screening, ADMET profiling, and molecular docking simulations to evaluate how the phytochemicals within *H. patens* leaves interact with NF-κB, COX-1, and CYP2E1. By establishing this initial computational framework, the study provides a clear, predictive foundation to guide subsequent in vivo and in vitro experimental validation.

2.0 MATERIALS AND METHODS

2.1 Plant Material collection and authentication

Leaves of *Hamelia patens* Jacq. were picked from the wild vegetation in the vicinity of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The plant material was authenticated by a taxonomist in the Department of Plant Science and Biotechnology at Michael Okpara University of Agriculture, Umudike. A voucher specimen was deposited in the Departmental Herbarium and assigned the voucher number MOUAU-PSB-2026-0847 on 4th March 2026.

2.2 Preparation of *Hamelia patens* leaf extract

The harvested leaves were carefully rinsed with clean water to remove any surface debris and air-dried at room temperature until a stable weight was achieved. These dried leaves were ground into a fine powder and kept in an airtight container until extraction. For the aqueous extraction process, 100 g of the pulverized leaf material was soaked in 1000 mL of distilled water for 24 hours with intermittent stirring. The mixture was first filtered through a clean muslin cloth and then passed through Whatman No. 1 filter paper. The resulting filtrate was concentrated using a rotary evaporator under reduced pressure at 40–45°C, and the concentrated aqueous extract was collected and stored under refrigerated conditions until use.

2.3 Preliminary phytochemical screening

Qualitative phytochemical screening of the *H. patens* leaf extract was carried out following standard analytical procedures (Harborne, 1973). The extract was evaluated to detect the presence of tannins, flavonoids, alkaloids, saponins, phytosteroids, and glycosides. Identification was determined by watching for characteristic colour modifications, precipitate development, or distinct frothing reactions.

2.4 Quantitative phytochemical analysis

Quantitative analysis of the prominent phytochemical groups relied on established gravimetric and spectrophotometric protocols. Quantification of alkaloids followed the Harborne (1973) technique, which involves precipitation via ammonium hydroxide after initial extraction using 10% ethanolic acetic acid. Total tannin concentration was measured utilizing the ferric chloride-potassium ferricyanide approach (Van-Burden & Robinson, 1981) against a standard curve of tannic acid. For flavonoids, evaluation followed the Boham and Kocipai-Abyazan (1974) workflow, utilizing repeated extractions in 80% aqueous methanol before gravimetrically weighing the dried residue. Saponin content was estimated according to the Obadoni & Ochuko (2001) procedure, which entails aqueous ethanol extraction, subsequent partitioning using diethyl ether and n-butanol, and final gravimetric calculation of the dried yield. Glycosides were determined using the method described by El-Olemy et al. (1994), based on the colour reaction produced with Baljet's reagent. Total phenolic content was determined using the Folin-Ciocalteu spectrophotometric method (Ainsworth & Gillespie, 2007).

2.5 GC-FID analysis of phytochemical constituents

The phytochemical constituents of the extract were characterized and quantified using gas chromatography coupled with flame ionization detection (GC-FID). Approximately 0.2 g of the extract was treated with 15 mL ethanol and 10 mL of 50% w/v potassium hydroxide and heated in a water bath at 60 °C for 3 h. Following the reaction, the mixture was transferred into a separatory funnel, while the reaction vessel was sequentially rinsed with ethanol, cold water, hot water and hexane, and the washings were combined with the extract. The combined extract was washed three times with 10 mL portions of 10% v/v aqueous ethanol. The solvent was evaporated, and the residue was reconstituted in 1000 µL pyridine, of which 200 µL was transferred into an autosampler vial for analysis. GC-FID analysis was performed using an Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, California, USA) equipped with a flame ionization detector and a RESTEK MXT-1 capillary column (15 m × 250 µm × 0.15 µm; Restek Corporation, Bellefonte, Pennsylvania, USA). Helium served as the carrier gas with the injector temperature maintained at 280°C. A 2 µL sample was introduced in splitless mode. The oven temperature, initially set at 200°C, was ramped up to 330°C at a rate of 3°C/min and subsequently held for 5 min. The detector temperature was kept at 320°C during operation. Identification of individual constituents relied on their specific chromatographic retention characteristics. Quantification was performed by calculating the peak area ratio of each identified compound relative to the internal standard, with final values expressed in µg/g or ppm.

2.6 *In silico* investigation

2.6.1 Selection and preparation of ligands

The GC-FID-identified phytochemicals that satisfied the abundance and literature-relevance criteria described below (not the broader phytochemical groups, i.e., individual named compounds) were carried forward from the preliminary phytochemical analysis into the computational (*in silico*) phase of the study. Based on their abundance and reported biological relevance to antioxidant, anti-inflammatory and hepatoprotective activities, tangeretin, resveratrol and luteolin were selected for molecular docking. Silymarin, a clinically used hepatoprotective agent, was included as the reference ligand. The molecular structures of the selected compounds were obtained from the PubChem database (National Center for Biotechnology Information, Bethesda, Maryland, USA) and prepared for docking by assigning appropriate bond orders, hydrogen atoms and partial charges.

2.6.2 Target protein selection and preparation

CYP2E1, COX-1 and NF-κB were selected as molecular targets because of their established involvement in xenobiotic bioactivation, inflammatory signalling and progression of hepatic injury. The three-dimensional crystal structures of the target proteins were retrieved from the RCSB Protein Data Bank (Research Collaboratory for Structural Bioinformatics, Piscataway, New Jersey, USA): human CYP2E1 in complex with indazole (PDB ID: 3E6I; resolution 2.20 Å), ovine COX-1 in complex with ibuprofen (PDB ID: 1EQG; resolution 2.61 Å), and the NF-κB p50 homodimer bound to κB DNA (PDB ID: 1NFK). Protein structures were prepared using AutoDockTools4 (Morris et al., 2009) by removing co-crystallised ligands, non-essential heteroatoms and water molecules, adding polar hydrogen atoms, and assigning Gasteiger partial charges.

2.6.3 Molecular docking analysis

Molecular docking was performed using AutoDock Vina (Trott & Olson, 2010). The docking search space (grid box) was centred on the co-crystallised ligand position of each target as follows: CYP2E1, centre (31.6, 25.6, 35.9), 25 × 25 × 25 Å; COX-1, centre (34.5, 28.2, 42.1), 25 × 25 × 25 Å; NF-κB, centre (21.4, 17.8, 30.2), 25 × 25 × 25 Å. The prepared ligands (tangeretin, resveratrol, luteolin and silymarin) were docked independently into each grid box. The binding affinity of each ligand was expressed as predicted binding energy (kcal/mol), with more negative values indicating a more thermodynamically favourable predicted pose. Predicted inhibition constants (Ki, µM) and root-mean-square deviation (RMSD, Å) between the top-ranked docking pose and the next-best pose from the same run were also recorded for comparison among the ligands.

2.6.4 Drug-likeness and toxicity prediction

Drug-likeness of the selected ligands was evaluated using SwissADME (Daina et al., 2017) based on Lipinski's Rule of Five, including molecular weight, hydrogen-bond acceptor and donor counts, lipophilicity (MLogP) and molecular refractivity. Molecular refractivity was also assessed as an additional physicochemical descriptor. Predicted acute oral toxicity and median lethal dose (LD_{50}) values were obtained using ProTox 3.0 (Charité–Universitätsmedizin Berlin, Berlin, Germany; https://tox.charite.de), which classifies compounds into toxicity classes I–VI according to the Globally Harmonized System (GHS) of Classification and Labelling of Chemicals based on 2D structural similarity to compounds of known LD_{50}. Compounds with acceptable physicochemical characteristics and favourable predicted toxicity profiles were considered to have greater potential for further pharmacological investigation.

2.7 Statistical analysis

Quantitative phytochemical data were expressed as mean ± standard deviation, while molecular docking and computational analyses were interpreted comparatively based on binding affinity, Ki, RMSD, drug-likeness and predicted toxicity parameters.

3.0 RESULTS

3.1 Phytochemical result

Table 1 shows the preliminary qualitative and quantitative phytochemical screening of the aqueous leaf extract of *Hamelia patens*. Qualitative analysis showed that alkaloids were highly present (+++), flavonoids, tannins, and phenolic compounds were moderately present (++), while saponins and glycosides were slightly present (+). Phytosteroids were absent (−). Quantitative phytochemical values are expressed as mean ± SD (n = 3). Alkaloids were the most abundant phytochemical, followed by flavonoids, phenolics, tannins, glycosides, and saponins.

Table **1.** Preliminary qualitative and quantitative phytochemical screening of *Hamelia patens* leaf extract

Phytocomponent	Qualitative inference	Quantitative amount (mg/100 g)
Alkaloid	+++	235.81 ± 1.47
Flavonoid	++	146.68 ± 0.91
Saponin	+	40.18 ± 1.06
Tannin	++	93.22 ± 2.01
Phenolic	++	95.89 ± 2.24
Glycoside	+	51.50 ± 1.88
Phytosteroid	−	–

Key: (+) Slightly present, (++) Moderately present, (+++) Highly present, (−) Absent.

3.2 GC-FID analysis of phytochemical constituents of Hamelia patens leaf extract

Table 2, showing the GC-FID analysis of the phytochemical constituents of *Hamelia patens* leaf extract, reveals the presence of 23 identified phytochemicals with a total quantified concentration of 380.52 ppm. Pinene was detected at the maximum concentration of 102.06 ppm. This was followed by descending amounts of limonene (60.97 ppm), tangeretin (42.42 ppm), isorhamnetin (29.68 ppm), resveratrol (28.80 ppm), lunamarin (25.66 ppm), and gallocatechin (25.11 ppm). Mid-range concentrations were recorded for hesperidin (12.79 ppm), decanal (11.46 ppm), luteolin (11.18 ppm), and ocimene (8.32 ppm). The remaining phytochemicals, including ellagic acid, vanillic acid, naringenin, octanal, myricetin, epicatechin, daidzein, genistein, apigenin, and naringin, were detected at relatively lower concentrations ranging from 0.18 to 6.06 ppm. The chromatogram obtained is shown in Figure 1.

Table **2.** GC-FID analysis of phytochemical constituents of *Hamelia patens* leaf extract

S/N	Phytochemical	Retention time (min)	Concentration (ppm)
1	Limonene	3.836	60.97
2	Pinene	4.267	102.06
3	Terpinene	5.176	2.76
4	Luteolin	6.358	11.18
5	Ocimene	6.519	8.32
6	Decanal	6.936	11.46
7	Ellagic acid	7.220	3.74
8	Vanillic acid	8.097	1.50
9	Naringenin	8.559	2.99
10	Octanal	8.868	2.73
11	Hesperidin	9.283	12.79
12	Isorhamnetin	9.467	29.68
13	Myricetin	10.140	1.08
14	Epicatechin	10.680	0.18
15	Daidzein	11.293	0.22
16	Genistein	11.661	0.24
17	Apigenin	13.007	0.35
18	Lunamarin	13.444	25.66
19	Gallocatechin	13.885	25.11
20	Resveratrol	14.061	28.80
21	Tangeretin	14.564	42.42
22	Catechin	15.064	6.06
23	Naringin	15.730	0.22

Total quantified phytochemicals: 380.52 ppm.

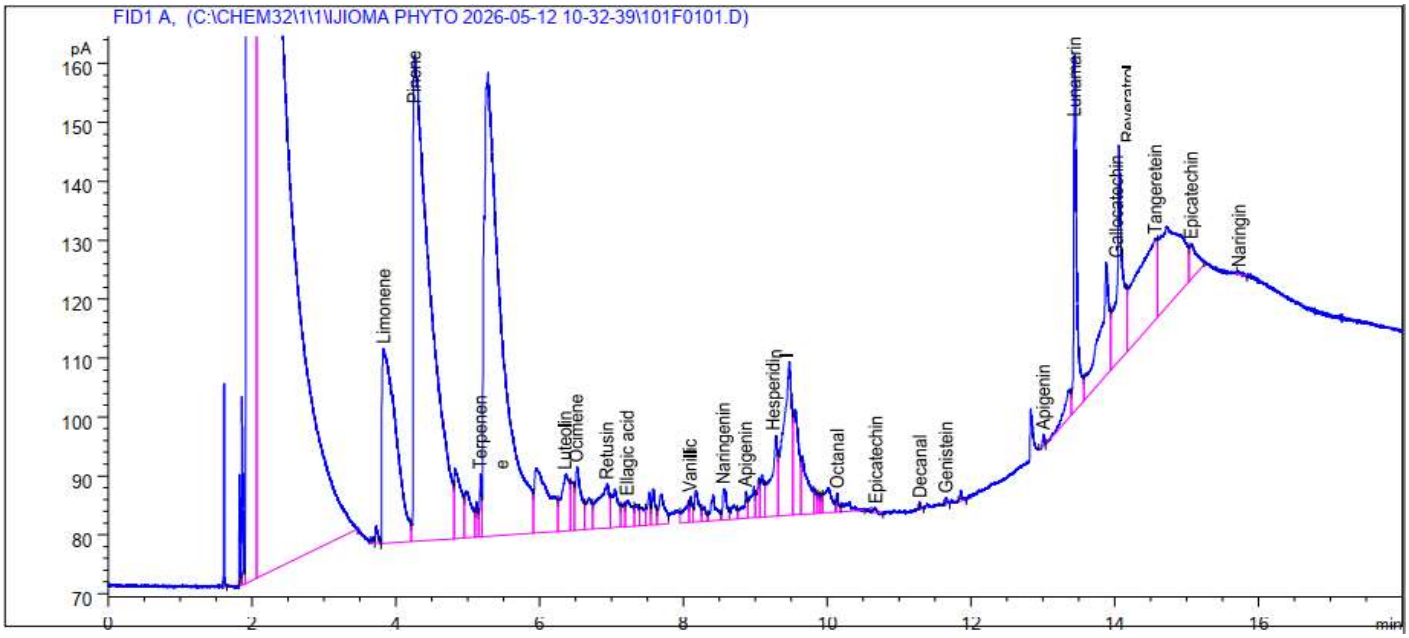

Figure **1.** GC-FID chromatogram of *Hamelia patens* leaf extract

3.3 Molecular docking

3.3.1 Drug-likeness of the selected ligands

Table 3, showing the drug-likeness analysis of the selected ligands, reveals that tangeretin, resveratrol, luteolin, and silymarin satisfied the criteria for drug-likeness based on Lipinski's Rule of Five. Tangeretin, resveratrol, and luteolin recorded no violations (0), while silymarin recorded one violation (1) but remained within the acceptable limit. All ligands had molecular weights below 500 g/mol, acceptable numbers of hydrogen bond donors and acceptors, lipophilicity values below 5, and molecular refractivity values within the recommended range of 40–130.

Table **3.** Drug-likeness analysis of the selected ligands

Compound	Mol. wt (g/mol)	HB acceptor	HB donor	Lipophilicity (MLogP)	Molecular refractivity	Lipinski violations
Tangeretin	372.37	7	0	0.63	100.38	0, Yes
Resveratrol	228.24	3	3	0.63	67.88	0, Yes
Luteolin	286.24	6	4	−0.031	76.01	0, Yes
Silymarin	482.44	10	5	−0.44	120.55	1, Yes

Acceptable ranges: molecular weight <500 g/mol; HB acceptor <10; HB donor ≤5; lipophilicity (MLogP) <5; molecular refractivity 40–130; Lipinski Rule of Five violations 0–4.

3.3.2 Toxicity and physicochemical properties of the selected ligands

Table 4, showing the toxicity and physicochemical properties of the selected ligands, reveals that tangeretin and luteolin were classified under toxicity class 5, with LD_{50} values of 5000 mg/kg and 3919 mg/kg, respectively, while resveratrol belonged to toxicity class 4 with an LD_{50} value of 1560 mg/kg. Tangeretin had the highest molecular weight (372.37 g/mol) and maximum tolerated daily dose (3.70 log mg/kg/day), followed by luteolin (286.24 g/mol, 3.59 log mg/kg/day) and resveratrol (228.24 g/mol, 3.19 log mg/kg/day).

Table **4.** Ligands, predicted toxicity class and physicochemical properties

Ligand	Toxicity class	LD50 (mg/kg)	Mol. wt (g/mol)	Daily MTD (log mg/kg/day)
Tangeretin	5	5000	372.37	3.70
Resveratrol	4	1560	228.24	3.19
Luteolin	5	3919	286.24	3.59

MTD = maximum tolerated dose. Toxicity class assigned according to the GHS/OECD acute oral toxicity classification scale (Classes 1–6, from most to least toxic), shown in Figure 2.



Figure **2.** GHS/OECD acute oral toxicity classification scale (Class 1–6) used to categorise the predicted toxicity of the selected ligands (adapted from the Globally Harmonized System of Classification and Labelling of Chemicals, as implemented in ProTox 3.0; Banerjee et al., 2018).

3.3.3 Molecular docking results of the selected ligands against CYP2E1

Table 5, showing the molecular docking results of the selected ligands against CYP2E1, reveals that silymarin exhibited the strongest binding affinity (−10.1 kcal/mol) with the lowest inhibition constant (Ki = 0.04) and an RMSD of 0. Among the phytochemical ligands, luteolin demonstrated the highest binding affinity (−9.1 kcal/mol) with a Ki of 0.21, whereas tangeretin and resveratrol showed equal binding affinities of −8.2 kcal/mol, each with a Ki of 0.96 and an RMSD of 0.

Table **5.** Molecular docking results of the selected ligands against CYP2E1

Ligand	Target protein	Binding affinity (kcal/mol)	Ki	RMSD
Silymarin	CYP2E1	−10.1	0.04	0
Tangeretin	CYP2E1	−8.2	0.96	0
Resveratrol	CYP2E1	−8.2	0.96	0
Luteolin	CYP2E1	−9.1	0.21	0

3.3.4 Molecular docking results of the selected ligands against COX-1

Table 6, showing the molecular docking results of the selected ligands against COX-1, reveals that luteolin exhibited the strongest binding affinity (−8.6 kcal/mol) with the lowest inhibition constant (Ki = 0.49) and an RMSD of 0. Silymarin showed a binding affinity of −7.7 kcal/mol with a Ki of 2.25, followed by tangeretin with a binding affinity of −7.5 kcal/mol and a Ki of 3.15. Resveratrol recorded the weakest binding affinity (−7.2 kcal/mol) and the highest Ki (5.22) among the ligands, while all ligands had an RMSD of 0.

Table **6.** Molecular docking results of the selected ligands against COX-1

Ligand	Target protein	Binding affinity (kcal/mol)	Ki	RMSD
Silymarin	COX-1	−7.7	2.25	0
Tangeretin	COX-1	−7.5	3.15	0
Resveratrol	COX-1	−7.2	5.22	0
Luteolin	COX-1	−8.6	0.49	0

3.3.5 Molecular docking results of the selected ligands against NF-κB

Table 7, showing the molecular docking results of the selected ligands against NF-κB, reveals that silymarin exhibited the strongest binding affinity (−7.5 kcal/mol) with the lowest inhibition constant (Ki = 3.15) and an RMSD of 0. Among the phytochemical ligands, luteolin demonstrated the highest binding affinity (−6.9 kcal/mol) with a Ki of 8.67, followed by tangeretin (−6.0 kcal/mol, Ki = 39.66) and resveratrol (−5.5 kcal/mol, Ki = 92.29). All ligands recorded an RMSD of 0, indicating stable docking conformations.

Table **7.** Molecular docking results of the selected ligands against NF-κB

Ligand	Target protein	Binding affinity (kcal/mol)	Ki	RMSD
Silymarin	NF-κB	−7.5	3.15	0
Tangeretin	NF-κB	−6.0	39.66	0
Resveratrol	NF-κB	−5.5	92.29	0
Luteolin	NF-κB	−6.9	8.67	0

4.0 DISCUSSION

The study herein aimed to investigate the hepatoprotective potential of the phytochemicals present in the extract of the leaves of *Hamelia patens* by carrying out the phytochemical profiling, drug-likeness evaluation and molecular docking with the important targets involved in the carbon tetrachloride (CCl_4) induced hepatotoxicity. The results offer computational evidence for the traditional medicinal application of *H. patens* for the treatment of liver diseases and can help identify specific bioactive ingredients that could be responsible for its therapeutic activity.

The preliminary phytochemical screening showed the presence of alkaloids, flavonoids, phenolics and tannins with alkaloids being the richest followed by flavonoids, phenolics and tannins. The phytochemical profile is similar to that previously reported for *H. patens*, where oxindole alkaloids, flavonoids and phenolic compounds are reported as main bioactive compounds (Ahmad et al., 2012; Perez-Meseguer et al., 2016). Pharmacologically, the presence of the predominance of alkaloids and polyphenolic compounds is important because they have been shown to exhibit hepatoprotective activities by their antioxidant, anti-inflammatory, and cytoprotective properties (Madrigal-Santillán et al., 2014). Hepatoprotective activity from CCl_4-induced damage in HepG2 cells was demonstrated in previous studies on *H. patens* butanol and ethyl acetate fractions which were rich in polyphenolic compounds, with the butanol fraction being more hepatoprotective than silibinin as a reference compound (Perez-Meseguer et al., 2016). Therefore, the amount of these phytochemical classes in the present study supports the rationale of hepatoprotection potential of the plant.

There were 23 phytochemicals identified by GC-FID, the most abundant ones being pinene and limonene as terpenes, followed by tangeretin (polymethoxylated flavone), isorhamnetin (flavonol) and resveratrol (stilbenoid). From these, tangeretin, luteolin and resveratrol were chosen as the molecules for further molecular docking due to their known biological activity and availability. These flavonoid-type compounds have been identified based on strong evidence that dietary flavonoids modulate oxidative stress, inflammation and metabolic pathways to show hepatoprotection (Ullah et al., 2020). Tangeretin, a polymethoxyflavone derived from citrus fruits, has been found to suppress oxidative stress and maintain the hepatocellular architecture by activation of the Nrf2/Keap1 pathway Arivazhagan & Subramanian (2015) and also to improve hepatic insulin sensitivity by inhibition of the MEK-ERK1/2 pathway (Guo et al., 2020). Resveratrol has been shown to protect against CCl_4-induced liver injury through mitophagy, mediated by the PINK1/Parkin pathway, by decreasing oxidative stress and by inhibiting the NF-κB pathway (Zhang et al., 2016; He et al., 2026). Luteolin shows hepatoprotective activity against different hepatotoxins such as aflatoxin B1 by activation of Nrf2 signalling pathway and by inhibiting apoptosis (Rajput et al., 2021; Zaki et al., 2023).

The drug-likeness assessment showed that tangeretin, resveratrol and luteolin met the Lipinski's Rule of Five criteria with no violations, but silymarin with one violation was still within acceptable limits. Favourable physicochemical properties for oral bioavailability such as appropriate molecular weight, lipophilicity and hydrogen bonding capacity were suggested by the compliance with these criteria (Lipinski et al., 2001). The toxicity profiling showed that tangeretin and luteolin were in toxicity class 5 (LD_{50} > 3000 mg/kg) and resveratrol was in class 4 (LD_{50} = 1560 mg/kg), indicating a favourable safety margin. These predictions open the door for the possible application of such compounds as therapeutic agents, but further testing in vivo is necessary. It is important to note that, although computational toxicity prediction can perform a useful preliminary screening, it does not always reflect all the complexity of biological systems, such as metabolic activation, tissue-specific effect and inter-individual variability (Alves et al., 2018).

Molecular docking results with CYP2E1 showed that the binding affinity of silymarin was found to be the highest among all the compounds docked (predicted binding affinity: −10.1 kcal/mol); luteolin showed a higher binding affinity of −9.1 kcal/mol, while tangeretin and resveratrol showed a comparable binding affinity of −8.2 kcal/mol. CYP2E1 is the major enzyme that bioactivates CCl_4 to trichloromethyl and trichloromethyl peroxyl radicals, which are responsible for the initiation of lipid peroxidation, oxidative stress and hepatocyte necrosis (Weber et al., 2003). CYP2E1-knockout mice have been shown to be significantly protected from CCl_4 hepatotoxicity, supporting the importance of this enzyme in liver injury (Wong et al., 1998). The high predicted interaction potential of luteolin with CYP2E1 makes it a pharmacologically significant interaction, and experimental research demonstrated that when silymarin was evaluated for its protection against liver injury induced by a variety of drugs, its protective effect involved reducing the expression and activity of CYP2E1 (Yang et al., 2022). It is worth noting, however, that the consequence of the binding of the molecule may not always be inhibitory; binding may have stimulatory or neutral effects depending on the binding mode of the molecule, and the conformational dynamics of the enzyme, which may not be captured by static docking techniques (Pagadala et al., 2017; Torres et al., 2019).

Luteolin showed the highest predicted binding affinity of −8.6 kcal/mol against COX-1, stronger than silymarin (−7.7 kcal/mol); this should not be read as a favourable finding without qualification. Because COX-1 is constitutively hepatoprotective rather than injurious in the liver, strong ligand occupancy of its active site is, on current evidence, more plausibly interpreted as a potential risk of functional inhibition than as a therapeutic mechanism. COX-1 has a constitutive hepatoprotective role in comparison to COX-2, which is usually involved in inflammatory processes, in maintaining the production of prostaglandin E2 (PGE2) for liver regeneration and homoeostasis (Xiao et al., 2015). Pharmacological inhibition of COX-1 or genetic deletion in mice has been shown to worsen CCl_4-induced acute liver damage, also linked to increased oxidative stress, inflammation, and impaired hepatic regeneration (Xiao et al., 2015). The predicted luteolin–COX-1 interaction should therefore be treated as a flag for functional follow-up rather than as evidence of hepatoprotection, and enzyme-activity assays (rather than binding affinity alone) are needed to determine whether luteolin activates, inhibits, or does not functionally affect COX-1 at physiologically relevant concentrations.

Silymarin exhibited the strongest predicted binding affinity during docking simulations with NF-κB at −7.5 kcal/mol. Among the plant constituents, luteolin (−6.9 kcal/mol), tangeretin (−6.0 kcal/mol), and resveratrol (−5.5 kcal/mol) also demonstrated appreciable binding affinities. This transcription factor, NF-κB, operates as a master regulator of fibrogenic and inflammatory pathways during CCl_4-mediated hepatic trauma, with its activation observed predominantly in hepatic stellate cells and Kupffer cells during chronic liver damage (Oakley et al., 2005). In a mouse model, severe liver fibrosis triggered by CCl_4 was attenuated by inhibition of NF-κB, which suppressed the activation of hepatic stellate cells and reduced pro-inflammatory cytokine output (Wang et al., 2014); this finding is drawn from rodent data and its extrapolation to human liver pathology should be made cautiously. The predicted molecular interaction between luteolin and NF-κB closely aligns with experimental data proving that luteolin acts as an inhibitor of NF-κB signalling, subsequently lowering inflammatory markers across diverse models of liver injury (Li & Gu, 2026). Similarly, resveratrol has been shown to attenuate CCl_4-induced liver fibrosis via inhibition of the NF-κB pathway and modulation of TGF-β/SMAD signalling (Zhang et al., 2016; Tanriverdi et al., 2016).

Among the phytochemicals evaluated, luteolin consistently showed the strongest predicted binding across all three targets by docking score; however, "favourable" here refers only to computed binding affinity, not to a confirmed hepatoprotective mechanism, the COX-1 result in particular could equally represent a risk of functional inhibition. Luteolin's affinity for CYP2E1 and NF-κB is more readily interpretable as consistent with hepatoprotection, while its interaction with COX-1 requires dedicated functional validation before any protective role is inferred. This finding is especially notable because luteolin, a natural compound, has been shown to possess hepatoprotective activities via various mechanisms in experimental studies, including antioxidant activity, modulation of drug-metabolizing enzymes and suppression of inflammatory signalling (Rajput et al., 2021). The current study used a hard (rigid) receptor docking approach that does not consider protein flexibility and induced-fit effects for the dynamical aspects of interactions between receptor and ligands in biological systems (Torres et al., 2019). Scoring functions in molecular docking are only approximate, modelling the actual free energy of binding and often do not take into account solvation effects, entropic contributions, or protein conformational changes (Chen, 2015). Consequently, the binding affinities reported here should be regarded as predictive indicators requiring validation through enzyme inhibition assays, cell-based studies, and in vivo experimentation.

It must be stressed that no CCl_4 induction, animal or cell-based experiment was performed in this study: all toxicity, drug-likeness and binding data reported here are computational predictions. The LD50 values in Table 4 are in silico estimates from ProTox 3.0 and have not been experimentally confirmed. The present findings should therefore be regarded as hypothesis-generating, and the absence of in vivo or in vitro validation is acknowledged as the principal limitation of this study.

Conclusion

Hamelia patens leaf extract is rich in phytochemicals with recognised antioxidant and hepatoprotective associations in the literature. Of the compounds evaluated, luteolin, tangeretin and resveratrol showed favourable predicted drug-likeness, acceptable predicted acute toxicity profiles, and appreciable predicted binding affinities for CYP2E1 and NF-κB, two targets directly implicated in CCl_4-induced hepatotoxicity; their predicted interaction with COX-1 is ambiguous and may reflect a risk of functional inhibition rather than protection. These in silico findings generate a testable hypothesis that select H. patens phytochemicals, particularly luteolin, interact with CYP2E1- and NF-κB-linked pathways relevant to hepatoprotection, but this hypothesis requires confirmation through enzyme-inhibition assays, cell-based studies and in vivo experimentation before any conclusion on hepatoprotective activity can be drawn.

Conflict of interest

The authors declare no conflict of interest.

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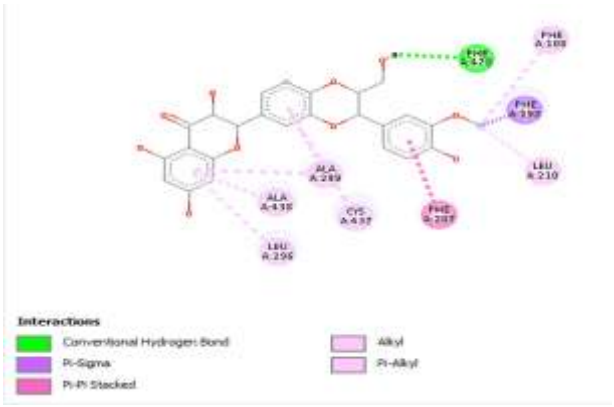
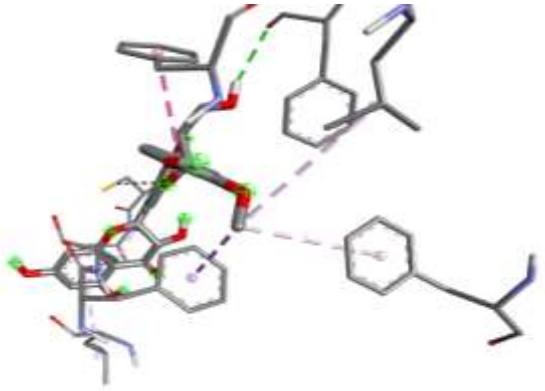
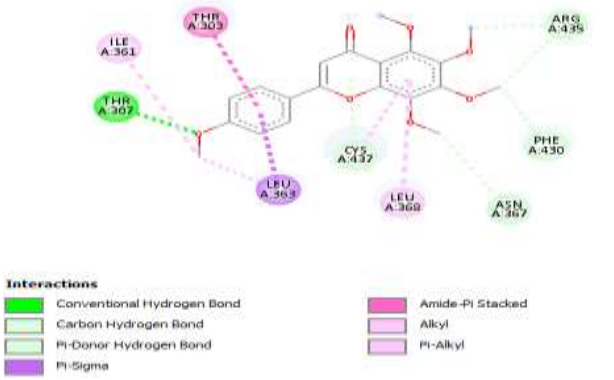
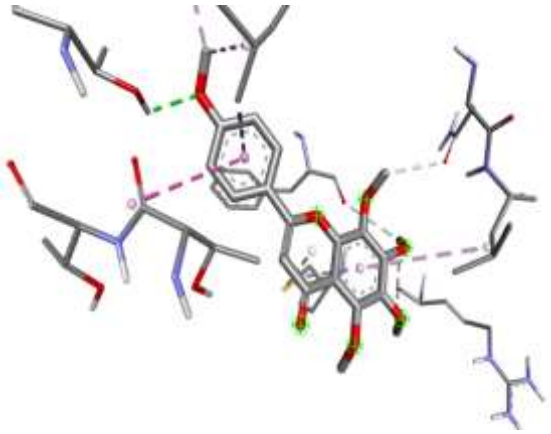


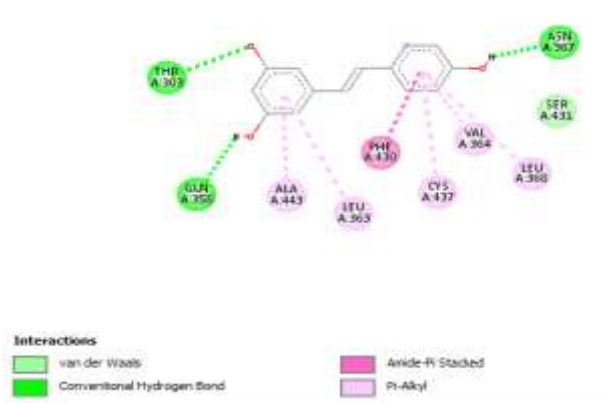


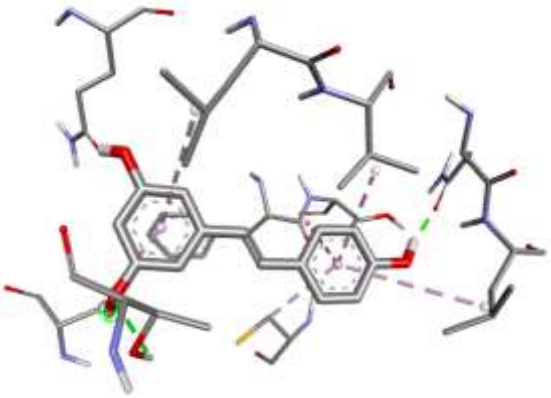
Silymarin (standard)



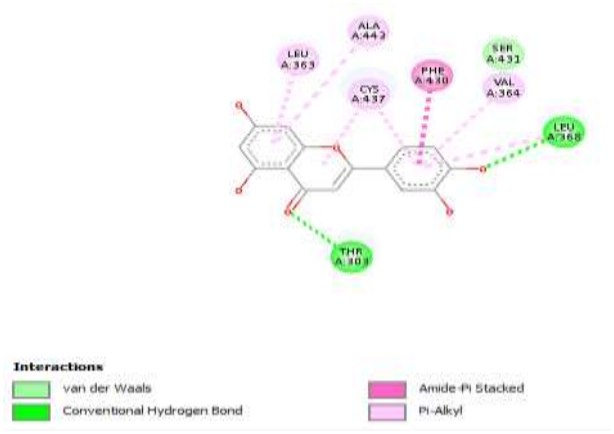


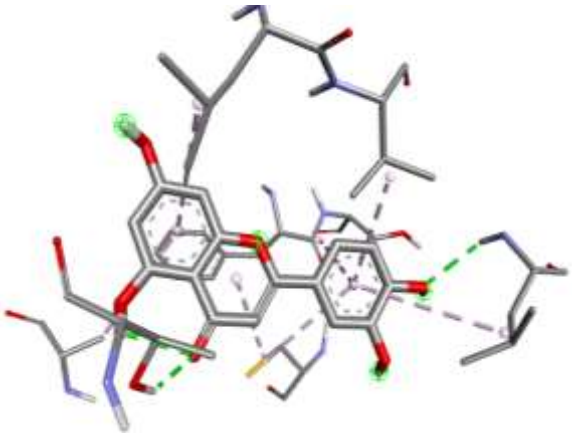
Tangeretin





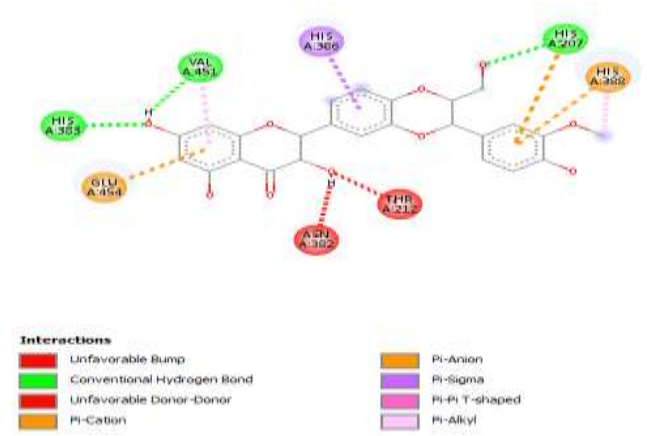
Resveratrol

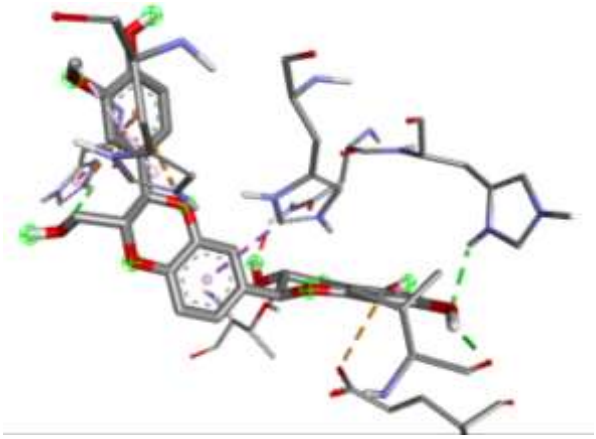




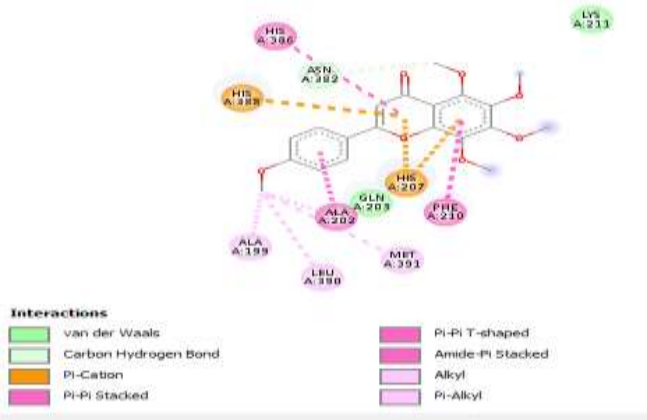
Luteolin

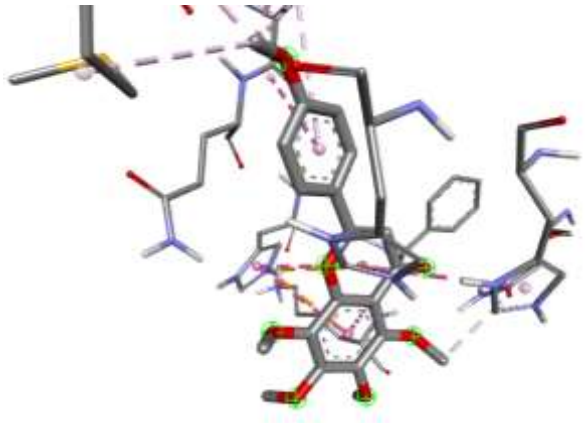
Figure **3.** Two-dimensional and three-dimensional binding poses of (A) silymarin, (B) tangeretin, (C) resveratrol and (D) luteolin in the active site of CYP2E1.



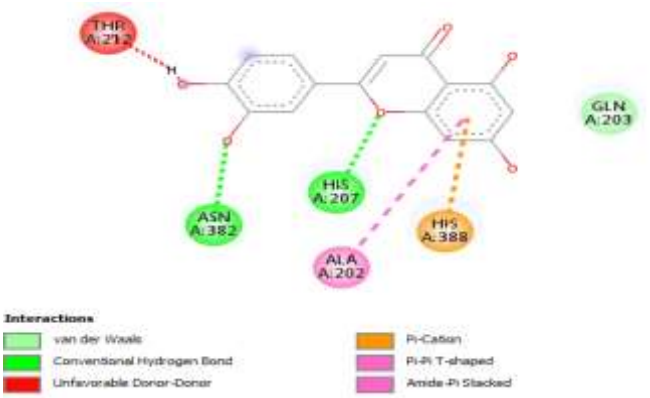


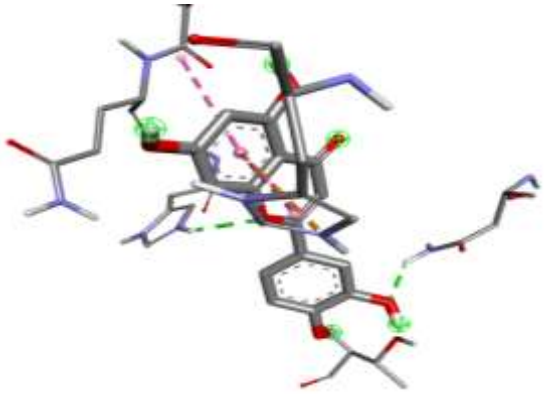
Silymarin



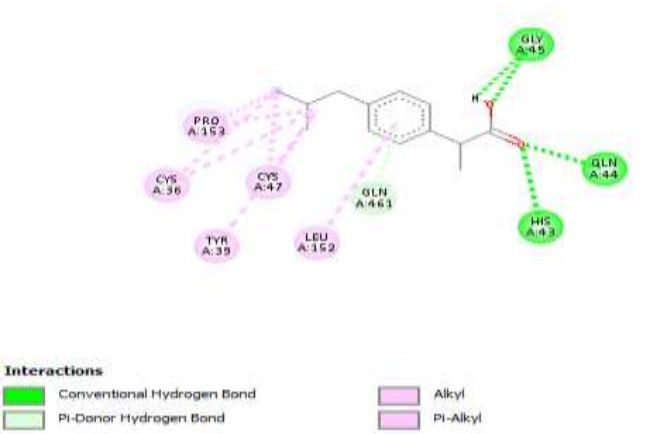


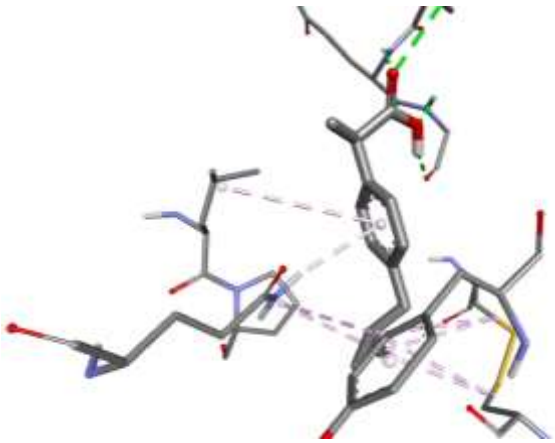
Tangeretin





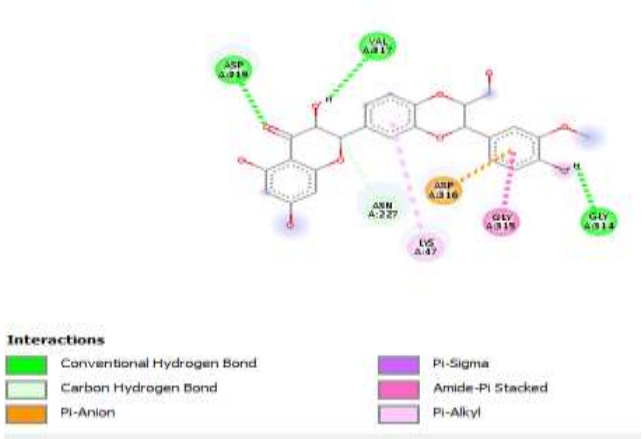
Luteolin

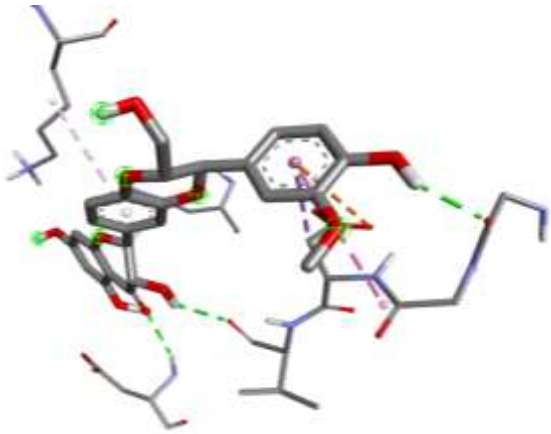




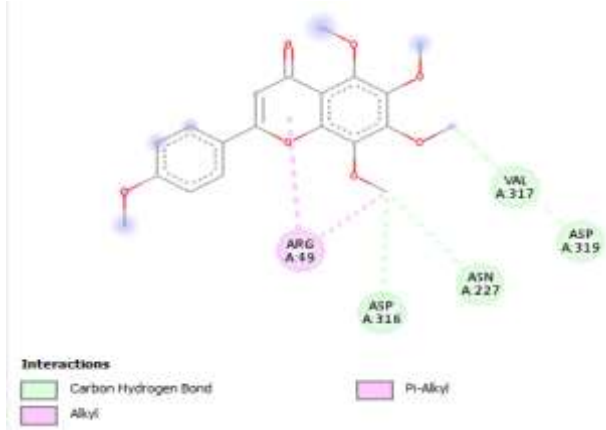
Ibuprofen (native ligand, redocking validation)

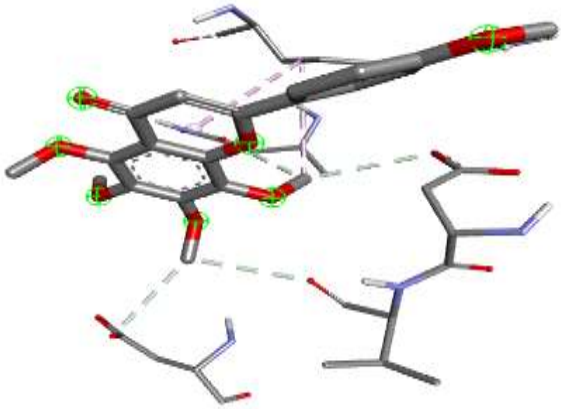
Figure **4.** Two-dimensional and three-dimensional binding poses of (A) silymarin, (B) tangeretin, (C) luteolin and (D) ibuprofen (native ligand, redocking validation) in the active site of COX-1.



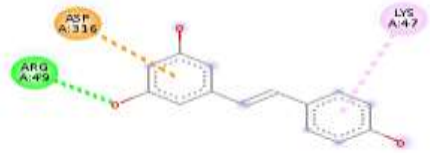


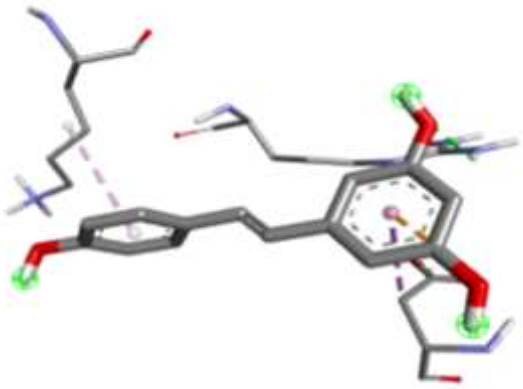
Silymarin



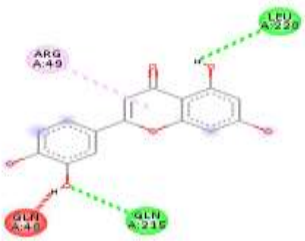


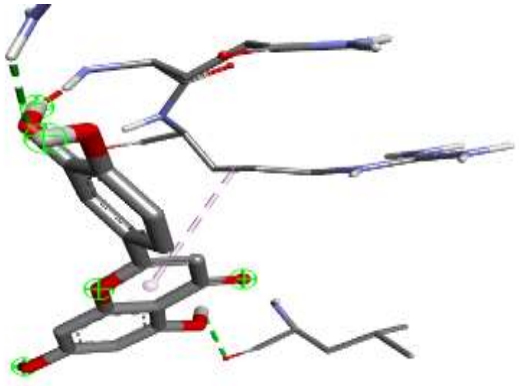
Tangeretin





Resveratrol





Luteolin

Figure **5.** Two-dimensional and three-dimensional binding poses of (A) silymarin, (B) tangeretin, (C) resveratrol and (D) luteolin at the p50 subunit of NF-κB.

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