INFORMATICS JOURNALS

Research Article

Journal of Natural Remedies
DOI: 10.18311/jnr/2026/54345

eISSN: 2320-3358 | Vol 26(7), 2026 | pp. 1781-1794

Phytochemical Profiling and Antioxidant Potential of *Cordia sinensis* Lam. Extract: Integration of Phenolic-Flavonoid Quantification, *In Vitro* Assays, and *In Silico* GPx Modulation

Pinkey Rawal[1], Kavitha Jayaseelan[1*] and Purnima Ashok[2]

[1]Department of Pharmaceutical Analysis, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur - 603203, Tamil Nadu, India; kavithaj@srmist.edu.in

[2]Department of Pharmacology, East West College of Pharmacy, Bengaluru - 560091, Karnataka, India

Abstract

Background: *Cordia sinensis* Lam. is traditionally employed in the management of oxidative stress and inflammation-associated conditions; however, systematic biochemical and mechanistic evaluation of its stem remains limited. **Aim:** This study aimed to evaluate the *in vitro* antioxidant activity of *C. sinensis* stem extracts and investigate their interaction with human Glutathione Peroxidase (GPx) using molecular docking. **Methods:** Ethanolic and aqueous stem extracts were prepared and quantitatively analysed for Folin-Ciocalteu and aluminium chloride tests, which were used to determine Total Phenolic Content (TPC) and Total Flavonoid Content (TFC). The *in vitro* antioxidant activity was evaluated, and molecular docking studies were performed against human glutathione peroxidase (GPx; PDB ID: 2P31) using AutoDock Vina v1.2.0. **Results:** The aqueous extract showed significantly higher TPC (36.20 ± 0.85 mg GAE/g) and TFC (158.79 ± 1.57 mg CE/g) than the ethanolic extract. Conversely, the ethanolic extract demonstrated superior antioxidant activity, with IC_{50} values of 6.94 ± 1.41 µg/mL (DPPH) and 7.47 ± 1.08 µg/mL (ABTS), comparable to quercetin. Docking analysis revealed stable binding interactions of major phenolic and flavonoid constituents. For instance, rutin exhibited a docking score of −7.7 kcal/mol against GPx. **Conclusion:** The findings demonstrate that *C. sinensis* stems possess substantial antioxidant potential driven by solvent-dependent phytochemical profiles.

Major Findings: The ethanolic extract of *C. sinensis* demonstrated antioxidant activity with IC_{50} values of 6.94 ± 1.41 µg/mL (DPPH) and 7.47 ± 1.08 µg/mL (ABTS). Among the evaluated phytoconstituents, rutin exhibited a docking score of −7.7 kcal/mol against GPx.

Keywords: Antioxidant Activity, *Cordia sinensis*, Molecular Docking, Phenolic Compounds, Phytochemical Profiling

(Article Chronicle: Article Received on: 09.01.2026; Revised on: 09.03.2026; Accepted on: 12.06.2026)

1. Introduction

Oxidative stress is a key biochemical process involved in the onset and advancement of several chronic and degenerative illnesses, such as diabetes mellitus, cardiovascular problems, neurodegenerative diseases, cancer, and inflammatory conditions[1]. This condition results from an imbalance between the overproduction of reactive oxygen and nitrogen species and the ability of endogenous antioxidant defense mechanisms to neutralise them, causing oxidative damage to lipids, proteins, and nucleic acids, along with the disruption of redox-sensitive signalling pathways[2]. Consequently, considerable research attention has been directed toward plant-derived antioxidants, which are capable of mitigating oxidative stress through complementary

*Author for correspondence

mechanisms such as direct free-radical scavenging, hydrogen or electron donation, interruption of radical chain reactions, metal chelation, and modulation of antioxidant enzymes[3].

Polyphenols are a significant class of plant secondary metabolites that enhance antioxidant activity[4]. Structural characteristics including the degree of conjugation, the number and position of hydroxyl groups, and the existence of glycosidic moieties critically influence their redox behaviour and biological effectiveness[5,6]. Among polyphenols, flavonoids are particularly significant due to their strong radical-scavenging capacity and their additional anti-inflammatory, antimicrobial, hepatoprotective, and cytoprotective properties[7,8]. Given the close relationship between polyphenolic composition and biological activity, quantitative evaluation of phenolic and flavonoid constituents, together with functional antioxidant assays, is essential for validating the therapeutic relevance of medicinal plants.

The genus *Cordia* (family *Boraginaceae*) comprises approximately 300 species widely distributed in tropical and subtropical regions and extensively utilised in traditional medicine. Several Cordia species have been reported to exhibit antioxidant, anti-inflammatory, wound-healing, antimicrobial, and hepatoprotective activities, largely attributed to their phenolic and flavonoid constituents[9,10]. *C. sinensis* Lam. is traditionally used to manage respiratory, gastrointestinal, inflammatory, and infectious disorders. Ethnobotanical reports further document the medicinal use of its roots and bark for stomach and chest ailments, malaria, and other traditional applications[11].

Despite its ethnomedicinal significance, scientific investigations on *C. sinensis* have primarily focused on leaves, roots, or fruits, while the stem remains comparatively underexplored. This gap is noteworthy, as stems often contain lignified tissues enriched with tannins, flavonoids, and structurally complex polyphenols that may substantially contribute to antioxidant activity. The present study aimed to quantitatively determine the total phenolic and flavonoid contents of aqueous and ethanolic extracts of *C. sinensis* stems, and to evaluate their antioxidant potential using DPPH and ABTS radical scavenging assays. Furthermore, to provide mechanistic insight, an *in silico* molecular docking approach was employed to assess the interaction of reported stem phytoconstituents with human glutathione peroxidase, a key endogenous antioxidant enzyme. By integrating phytochemical profiling, *in vitro* antioxidant evaluation, and computational analysis, this work seeks to establish a biochemical and molecular framework supporting the antioxidant potential and ethnomedicinal relevance of *C. sinensis* stems.

2. Materials and Methods

2.1 Collection and Authentication of Plant Material

The stems of *C. sinensis* Lam. were collected from the Mandwaria region of Sirohi District, Rajasthan, India. The plant material was taxonomically identified and authenticated by Dr. V. Rama Rao, Research Officer (Botany), Central Ayurveda Research Institute, Bengaluru, India. A voucher specimen (RRCBI-mus470) was prepared and deposited in the institute's herbarium for future reference. Following authentication, the collected stems were shade-dried at ambient temperature and subsequently pulverised into a coarse powder using a mechanical grinder. The powdered material was stored in an airtight container under dry conditions until further use[12]. All chemicals and reagents utilised were of analytical grade. Fresh solutions and working standards were prepared immediately prior to analysis to ensure analytical accuracy.

2.2 Preparation of Extracts

Dried stem powder of *C. sinensis* (100 g) was extracted using two solvent systems to obtain ethanolic and aqueous extracts. For the ethanolic extract, the powdered material was macerated with 70% ethanol in a 1:10 (w/v) ratio for 72 h at room temperature with intermittent shaking to facilitate solvent penetration. The extract underwent filtration using Whatman No. 1 filter paper, followed by concentration of the filtrate under reduced pressure with a rotary evaporator at a temperature of 40 ± 2°C, resulting in a semisolid extract[13]. The aqueous extract was prepared following the same extraction, filtration, and concentration protocol, using distilled water as the extraction solvent. The dried extracts were stored in airtight containers

at 4°C until further phytochemical and antioxidant analyses[14].

2.3 Qualitative Analysis

Qualitative phytochemical screening of aqueous and ethanolic extracts of *C. sinensis* stems was performed using standard procedures[12].

2.4 Determination of Total Phenolic Content (TPC)

The content of total phenolic compounds was determined using the Folin-Ciocalteu procedure. The sample (250 µg/mL) or standard gallic acid solution (10-100 µg/mL) 0.2 mL was mixed with 0.5 mL of the Folin-Ciocalteu reagent (diluted 1:10 with deionised water) and 0.8 mL of sodium bicarbonate solution (7.5% w/v). The mixture was allowed to stand at room temperature (28 ± 2°C) for 30 min with intermittent shaking. The absorbance of the mixture was measured at 765 nm using a UV-vis spectrophotometer (PerkinElmer, USA). The content of total phenolic compounds was calculated as mean ± SD (n = 3) and expressed as milligrams of Gallic Acid Equivalent (GAE) in 1 g of the extract and dried powder[15].

2.5 Determination of Total Flavonoid Content (TFC)

The total flavonoid content was assessed utilising the $AlCl_3$ colorimetric method, employing catechin as the reference standard, in accordance with previously established protocols[16,17]. About 4 mL of distilled water was combined with 1 mL of the extract solution (0.1 mg/mL) or catechin standard solution (100-600 mg/L), and then 0.3 mL of 5% $NaNO_2$ was added. After 5-minute incubation at room temperature, 0.3 mL of a 10% AlCl3 solution was added, and the reaction mixture was allowed to stand for 6 minutes. Following this, 2 mL of 1M NaOH was added, and the total volume was adjusted to 10 mL with distilled water. The solution underwent vortexing, followed by immediate measurement of absorbance at 510 nm utilising a UV-Visible spectrophotometer, with a reagent blank serving as the reference. The total flavonoid content was determined using the catechin calibration curve and is presented as milligrams of catechin equivalents per gram of extract (mg CE/g). All measurements were conducted in triplicate.

2.6 *In Vitro* Antioxidant Activity

2.6.1 DPPH Free Radical Scavenging Assay

The antioxidant capacity of the ethanolic and aqueous stem extracts of *C. sinensis* was assessed through the DPPH radical scavenging method, with quercetin serving as a standard antioxidant[18]. A 0.1 mM DPPH solution was freshly prepared in methanol and stored in a light-protected environment to avoid degradation. Various concentrations of the extract, specifically 50, 100, 200, 250, and 300 µg/mL, were prepared in methanol. 1 mL of each concentration was combined with 3 mL of the DPPH solution and mixed thoroughly to ensure proper interaction. The reaction mixture was maintained at a temperature of 25 ± 2 °C in the absence of light for a duration of 30 minutes to ensure the reaction reached completion[19]. After incubation, the reduction in absorbance was measured at 517 nm utilising a UV-Visible spectrophotometer, with methanol acting as the blank control. The calculation of the percentage of DPPH radical scavenging activity was performed using equation 1, with results presented as mean values derived from triplicate determinations[20].

$$Percentage\ Inhibition = \left(\frac{Acontrol - Aextract}{Acontrol}\right) \times 100 \qquad (1)$$

where, $A_{control}$ and $A_{extract}$ represent the absorbance of the control and extract, respectively.

2.6.2 ABTS Radical Cation Assay

The antioxidant capacity of the ethanolic and aqueous stem extracts of *C. sinensis* was assessed using the ABTS radical cation decolorisation assay, incorporating minor modifications to the established methodology[21]. A 7 mM ABTS stock solution was combined with 2.45 mM potassium persulfate to produce the ABTS radical cation. The reaction mixture was maintained in a dark environment at room temperature for a duration of 12 to 16 hours to facilitate the development of a stable blue-green chromophore. Before conducting the analysis, the ABTS solution underwent dilution with ethanol to achieve an absorbance of 0.70 ± 0.02 at 734 nm. Various concentrations of the extract (50, 100, 200, 250, and 300 µg/mL) were prepared, and 1 mL of each concentration was combined with 3 mL of the ABTS working solution. The samples were subjected to incubation in the absence of light at a controlled temperature of 25 ± 2 °C for a duration of 30 minutes.

Following this, absorbance measurements were taken at 734 nm utilising a UV-Visible spectrophotometer, employing ethanol as the blank control. The percentage inhibition of the ABTS radical was determined using equation 2, and all experiments were performed in triplicate to ensure the reliability of the results.

$$Percentage\ Inhibition = \left(\frac{Acontrol - Aextract}{Acontrol}\right) \times 100 \qquad (2)$$

where, $A_{control}$ and $A_{extract}$ represent the absorbance of the control and extract, respectively.

2.7 *In Silico* Study

Phytoconstituents reported from *C. sinensis* were initially retrieved from the IMPPAT 2.0 database[22]. This was followed by manual curation to remove duplicate entries, fatty acids, amino acids, long-chain hydrocarbons, and their acid derivatives; additionally, a limited number of phytochemicals documented in the literature but absent from the database were incorporated to ensure comprehensive chemical coverage[23]. Finally, the list included 16 phytoconstituents and one reference drug (quercetin) for docking.

All selected ligands were subjected to energy minimisation using the MMFF94 force field implemented in Open Babel, and saved in appropriate formats for docking[24,25]. The three-dimensional crystal structure of human glutathione peroxidase (PDB ID: 2P31, Resolution: 2.00 Å)[26] was retrieved from the Protein Data Bank and prepared using AutoDock Tools v1.5.7[27]. Human glutathione peroxidase (GPx) was selected as the target protein because it is a key enzyme in the cellular antioxidant defence system, responsible for detoxifying hydrogen peroxide and lipid hydroperoxides and maintaining redox homeostasis. Interaction of phytoconstituents with GPx may support enzymatic antioxidant activity and complement direct free-radical scavenging, providing a mechanistic basis for antioxidant potential[28,29].

The process entailed the removal of co-crystallised ligands and water molecules, the addition of polar hydrogens, and the allocation of Kollman charges[30]. The active binding pocket was identified using PrankWeb, a structure-based ligand-binding site prediction server[31]. The process of molecular docking was conducted utilising AutoDock Vina version 1.2.0[32]. The grid box is positioned with its centre at coordinates center_x = −13.8909, center_y = −10.1940, and center_z = −6.220. It has dimensions of size_x = 40, size_y = 40, and size_z = 40, ensuring complete coverage of the active site. The docking parameters were set with an energy range of 4 kcal/mol and an exhaustiveness value of 8. The top-ranked binding conformations were selected based on docking scores and interaction profiles. The ligand-protein interactions were visualised using PyMOL v3.1 to examine ligand orientation and conformation.

2.8 Statistical Analysis

All experiments were conducted in triplicate, and the results were presented as mean ± SD. The statistical analysis was conducted using GraphPad version 10.0. Comparative statistical analyses of aqueous and ethanolic extracts were conducted employing Student's t-test. A $p<0.05$ was deemed statistically significant.

3. Results and Discussion

3.1 Qualitative Analysis

Preliminary phytochemical screening of aqueous and ethanolic extracts of *C. sinensis* Lam. stems confirmed the presence of major polyphenolic constituents, including phenolics, flavonoids, and tannins, along with trace levels of saponins and glycosides. The relative distribution of these constituents across the extracts is presented in Table 1. The predominance of polyphenolic compounds supports further quantitative evaluation of total phenolic and flavonoid content and their contribution to antioxidant activity.

3.2 TPC

TPC analysis revealed a clear variation between the solvent extracts of *C. sinensis* stems. Quantification based on the gallic acid calibration curve showed that the aqueous extract possessed a higher phenolic concentration, with a TPC of 36.20 ± 0.85 mg GAE/g extract, compared to 30.67 ± 1.20 mg GAE/g extract observed for the ethanolic extract (Table 2). The difference in total phenolic content between the aqueous and ethanolic extracts was statistically significant ($p<0.05$).

3.3 TFC

The assessment of flavonoid levels in the stem extracts of *C. sinensis* was conducted utilising the aluminium

Table 1. Preliminary phytochemical screening of aqueous and ethanolic extracts of *C. sinensis* Lam. stems

Phytochemical constituent	Tests	Aqueous extract	Ethanol extract
Carbohydrates	Molisch's test	+	+
	Fehling's test	+	+
	Benedict's test	+	+
Alkaloids	Mayer's test	-	-
	Dragendorff's test	-	-
	Wagner's test	-	-
	Hager's test	-	-
Proteins and free amino acids	Biuret test	+	+
	Millon's test	+	+
	Xanthoprotein test	+	+
	Ninhydrin test	+	+
Phytosterols	Salkowski's test	-	++
	Liebermann-Burchard's test	-	++
Saponins	Foam test	+	-
Glycosides	Modified Borntrager's test	+	+
	Legal's test	-	-
Tannins	Alkaline reagent test	+++	+++
	Bromine water test	+++	+++
	Potassium dichromate test	+++	+++
Phenolics	Iodine test	+++	+++
	Ferric chloride test	+++	+++
	Silver nitrate test	++	++
Flavonoids	Lead acetate test	+++	+++
	Ferric chloride test	+++	+++
	Shinoda test	+++	+++

+++ High, ++ Moderate, + Slight, - Absent

Table 2. TPC of *C. sinensis* extracts

S. No.	Extract type	TPC (mg GAE/g extract, mean ± SD)
1	Aqueous	36.20 ± 0.85
2	Ethanolic	30.67 ± 1.20

Table 3. TFC of *C. sinensis* extracts

S. No.	Extract type	TFC (mg CE/g extract, mean ± SD)
1	Aqueous	158.79 ± 1.57
2	Ethanolic	132.54 ± 1.30

chloride colorimetric method, with catechin serving as the external standard. Quantitative estimation derived from the catechin standard curve indicated a greater accumulation of flavonoids in the aqueous extract relative to the ethanolic extract. The TFC was determined as 158.79 ± 1.57 mg CE/g extract for the aqueous fraction and 132.54 ± 1.30 mg CE/g extract for the ethanolic fraction (Table 3). The difference in total flavonoid content between the aqueous and ethanolic extracts was found to be statistically significant ($p<0.05$)

3.4 Antioxidant Activity

3.4.1 DPPH Radical Scavenging Assay

The aqueous and ethanolic stem extracts of *C. sinensis* demonstrated a concentration-dependent enhancement in DPPH radical scavenging activity across the tested concentration range of 50-300 µg/mL (Table 4 and Figure 1). The ethanolic extract exhibited stronger scavenging effects compared to the aqueous extract across all concentrations. At 300 µg/mL, the

ethanolic extract produced 88.75 ± 0.23% inhibition, whereas the aqueous extract showed 77.38 ± 0.24% inhibition. Under the same conditions, the reference quercetin demonstrated 95.56 ± 0.18% inhibition.

The DPPH analysis revealed that the ethanol extract exhibited significant activity, with an IC_{50} value of 6.94 ± 1.41 µg/mL, comparable to quercetin (IC_{50} = 8.09 ± 1.17 µg/mL). Conversely, the aqueous extract demonstrated markedly diminished activity, with an IC_{50} of 31.80 ± 1.81 µg/mL, necessitating nearly five times the concentration to achieve similar inhibition levels.

3.4.2 ABTS Radical Cation Assay

Assessment of *C. sinensis* stem extracts using the ABTS radical cation assay revealed clear differences in scavenging efficiency between the two solvent systems. As shown in Table 5 and Figure 2, ABTS inhibition increased progressively with increasing extract concentration for both aqueous and ethanolic samples. At 50 µg/mL, the aqueous and ethanolic extracts exhibited inhibition values of 55.45 ± 0.12% and 79.10 ± 0.07%, respectively, which increased to 81.30 ± 0.07% and 90.18 ± 0.07% at 300 µg/mL. Under the same conditions, the reference antioxidant quercetin showed 97.03 ± 0.19% inhibition at 300 µg/mL.

The ABTS assay revealed that the ethanol extract exhibited significant radical scavenging capacity, with an IC_{50} value of 7.47 ± 1.08 µg/mL, which is comparable to the activity of quercetin (IC_{50} = 8.29 ± 1.37 µg/mL). The aqueous extract demonstrated reduced efficacy, presenting an IC_{50} of 30.84 ± 2.04 µg/mL, thereby

Table 4. DPPH radical scavenging activity of aqueous extract, ethanolic extract, and quercetin at different concentrations (mean ± SD, n = 3)

Concentration (µg/mL)	Aqueous extract	Ethanol extract	Quercetin
	% inhibition (Mean ± SD)	% inhibition (Mean ± SD)	% inhibition (Mean ± SD)
50	52.27 ± 0.3	78.55 ± 0.18	83.99 ± 0.3
100	61.86 ± 0.18	80.93 ± 0.13	87.34 ± 0.18
150	65.4 ± 0.31	82.78 ± 0.06	90.76 ± 0.18
200	70.41 ± 0.12	84.31 ± 0.18	92.09 ± 0.18
250	73.67 ± 0.07	85.97 ± 0.2	93.59 ± 0.12
300	77.38 ± 0.24	88.75 ± 0.23	95.56 ± 0.18

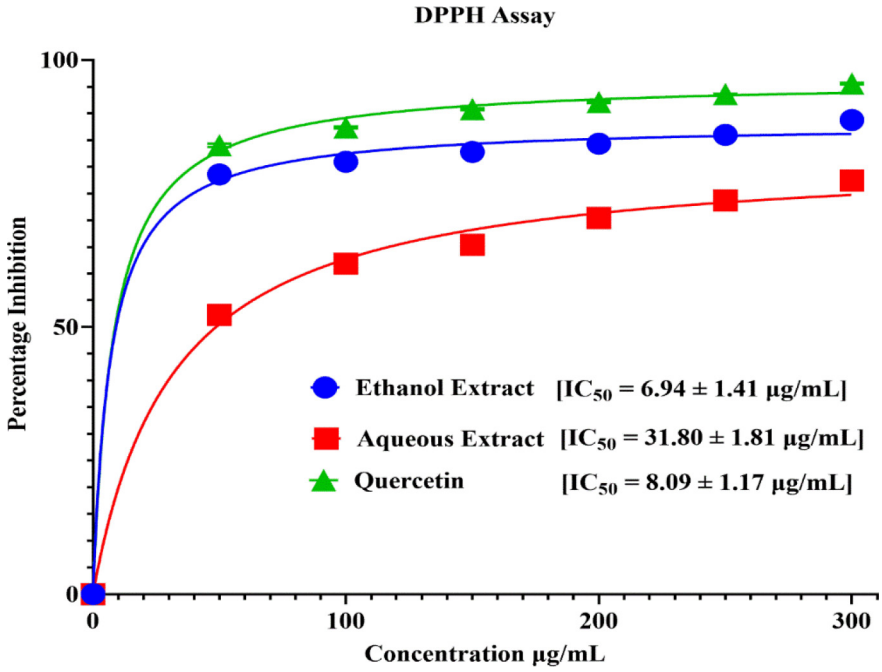


Figure 1. DPPH radical scavenging activity of *C. sinensis* stem extracts. Values represent mean ± SD.

Table 5. ABTS radical scavenging activity of aqueous extract, ethanolic extract, and quercetin at different concentrations (mean ± SD, n = 3)

Concentration (µg/mL)	Aqueous extract	Ethanol extract	Quercetin
	% inhibition (Mean ± SD)	% inhibition (Mean ± SD)	% inhibition (Mean ± SD)
50	55.45 ± 0.12	79.10 ± 0.07	85.02 ± 0.12
100	64.25 ± 0.19	81.17 ± 0.07	88.40 ± 0.07
150	69.24 ± 0.19	83.24 ± 0.12	91.41 ± 0.07
200	73.05 ± 0.07	85.02 ± 0.12	92.97 ± 0.07
250	77.15 ± 0.12	87.01 ± 0.07	95.30 ± 0.12
300	81.30 ± 0.07	90.18 ± 0.07	97.03 ± 0.19

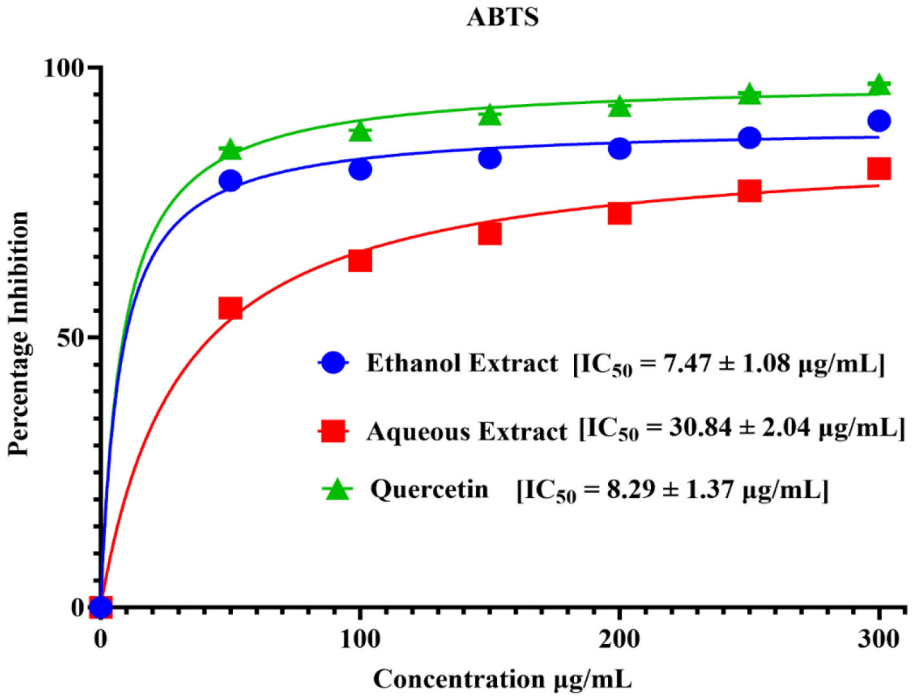


Figure 2. ABTS radical scavenging activity of *C. sinensis* stem extracts. Values represent mean ± SD.

underscoring the influence of solvent on the extraction of antioxidant compounds.

3.5 *In Silico* Result Analysis

PrankWeb-predicted binding pocket ranked as the top site (Rank 1) with a pocket score of 2.70 and probability of 0.082. The pocket comprises ten key residues (chain B): 32, 36, 42, 43, and 105-115 (Figure 3), which were subsequently used to define the docking grid for molecular docking simulations.

3.5.1 Molecular Docking Analysis

Molecular docking was employed to rationalise the antioxidant potential of *C. sinensis* stem phytoconstituents by evaluating their docking scores and interaction profiles against human glutathione peroxidase (GPx), a key enzyme involved in cellular redox homeostasis. The docking protocol successfully positioned all ligands within the catalytic pocket of GPx, indicating appropriate grid selection and binding site relevance.

3.5.2 Docking Scores Ranking and Comparison with the Reference Drug

Among the investigated compounds, rutin exhibited the highest binding affinity with a docking score of −7.7 kcal/mol, outperforming the reference antioxidant quercetin (−6.7 kcal/mol) by ~1.0 kcal/mol, suggesting

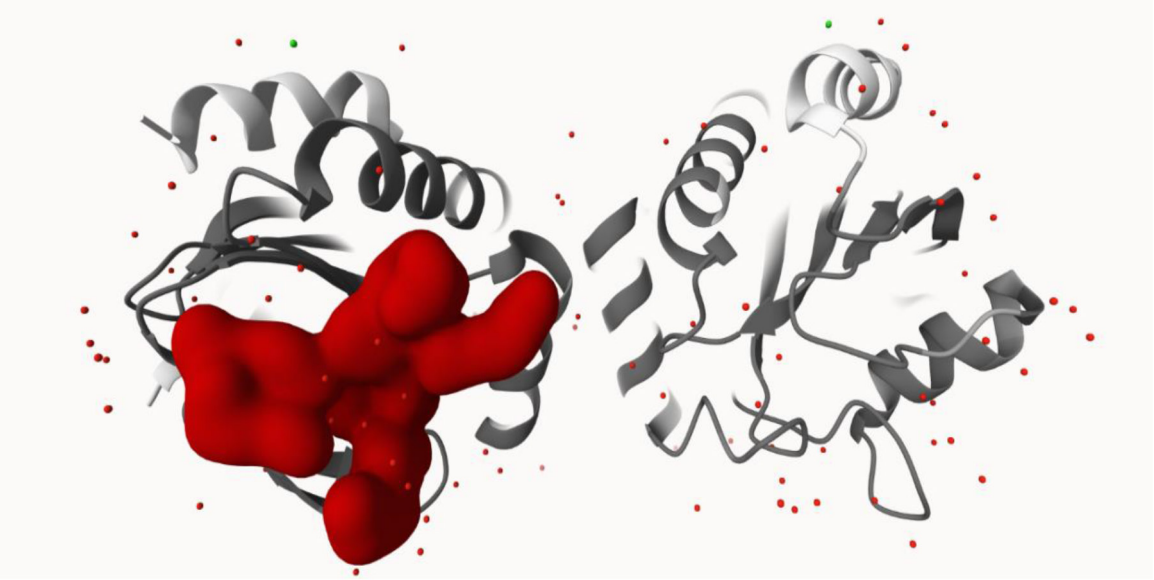


Figure 3. Binding site amino acids and docking grid box around the GPx cavity.

enhanced stabilisation within the GPx active site (Table 6). Other top-ranking phytoconstituents included β-amyrin (−7.1 kcal/mol) and floridanine (−7.0 kcal/mol), both demonstrating docking scores comparable to or superior to quercetin.

Moderate affinity was observed for apigenin (−6.8 kcal/mol), quercetin-3-O-β-D-glucopyranoside (−6.6 kcal/mol), scutellarein (−6.5 kcal/mol), and lupeol (−6.5 kcal/mol), whereas simple phenolic acids and sugars such as caffeic acid, protocatechuic acid, and monosaccharides displayed weaker binding (−5.1 to −5.7 kcal/mol), reflecting limited structural complementarity and fewer stabilising interactions.

3.5.3 Interaction Pattern and Residue Involvement

The interaction analysis revealed that Arg-rich and polar residues dominate GPx-ligand recognition, consistent with the redox-active and polar nature of the enzyme pocket. The reference compound quercetin formed stable hydrogen bonds with Arg65, Thr107, and Arg106, residues located near the catalytic region and commonly associated with substrate stabilisation.

Rutin, despite forming a single conventional hydrogen bond with Asn80, achieved a superior docking score due to its larger molecular framework, enabling extensive hydrogen bonding networks and hydrophobic surface complementarity, as evident from its deep insertion into the binding cavity (Figure 4A). This highlights that overall interaction surface and molecular size can outweigh the absolute number of hydrogen bonds.

β-Amyrin, a triterpenoid, demonstrated a strong docking score despite forming only one hydrogen bond with Arg34, suggesting that hydrophobic interactions and steric fitting play a dominant role in its stabilisation (Figure 4B). This behaviour contrasts with polyphenols, which primarily rely on hydrogen bonding.

Flavonoids such as apigenin, kaempferol, scutellarein, and quercetin glycosides exhibited multiple hydrogen bond interactions involving Arg106, Gln69, Asn32, and Asn80 (Figure 4C), residues repeatedly implicated across high-affinity ligands, underscoring their importance in GPx binding.

This study illustrates a distinct impact of solvent polarity on the extraction efficiency of phenolic compounds from *C. sinensis* stems. Phenolic compounds represent a significant and biologically active category of metabolites derived from plants, and they are well-documented for their positive impacts on human health[33]. The higher total phenolic content observed in the aqueous extract can be attributed to the strong polarity, higher dielectric constant, and extensive hydrogen-bonding capability of water, which collectively enhance solvent diffusion into lignified stem matrices and facilitate the solubilisation of hydrophilic phenolic acids and polyhydroxylated compounds.

Table 6. Molecular docking results of *C. sinensis* phytoconstituents against human glutathione peroxidase (PDB ID: 2P31) protein

S. No.	Phytoconstituents	Docking score (kcal/mol)	Conventional hydrogen bonding
1	Beta-D-Xylopyranose	-5.3	Arg105, Met114
2	Beta-Amyrin	-7.1	Arg34
3	Beta-Sitosterol	-6.3	Arg34
4	D-Galacturonic Acid	-5.7	Arg105, Lys42
5	L-Rhamnose	-5.1	Arg105, Met114, Asn32
6	Floridanine	-7.0	Arg65, Thr107, Gln62, Gln69, Tyr108
7	Methyl Rosmarinate	-6.1	Arg65, Gln69, Asn32
8	Rosmarinic Acid	-6.2	Arg65, Gln69, Thr107
9	Rutin	-7.7	Asn80
10	Quercetin-3-O-β-D-Glucopyranoside	-6.6	Lys42, Tyr43, Gln71
11	Quercetin/Reference	-6.7	Arg65, Thr107, Arg106
12	Kaempferol	-6.4	Arg34, Asn32, Arg106, Asn80, Ser111
13	Scutellarein	-6.5	Arg105, Phe112, Asn32
14	Apigenin	-6.8	Arg106, Gln69
15	Caffeic Acid	-5.2	Arg106, Asp61
16	Protocatechuic Acid	-5.7	Arg106, Asp95
17	Lupeol	-6.5	-

Similar observations have been widely reported for phenolic-rich medicinal plants, where aqueous or highly polar solvents preferentially extract low-molecular-weight phenolics and glycosylated derivatives[34,35]. These findings suggest that the stem tissue of *C. sinensis* serves as a metabolically active reservoir of water-soluble phenolics, which may contribute to its ethnomedicinal relevance and protective biological functions.

Flavonoid extraction also exhibited pronounced solvent selectivity. The higher total flavonoid content detected in the aqueous extract indicates a predominance of polar flavonoid glycosides, which are known to exhibit greater affinity toward high-polarity solvents. In contrast, ethanol tends to favour the extraction of less-polar flavonoid aglycones, O-methylated derivatives, and other moderately polar polyphenols[36,37]. This solvent-dependent partitioning of flavonoid subclasses has been consistently reported across several medicinal plant species and is strongly influenced by the degree of hydroxylation and glycosylation of the flavonoid backbone[38]. From a pharmacological perspective, flavonoid glycosides generally demonstrate improved aqueous solubility and intestinal transport, whereas aglycones often show higher membrane permeability and stronger intrinsic antioxidant properties due to the availability of free hydroxyl groups[39].

Interestingly, despite the higher total phenolic and flavonoid contents observed in the aqueous extract, the ethanolic extract exhibited superior antioxidant activity in both DPPH and ABTS assays. This apparent discrepancy underscores that antioxidant efficacy is not determined solely by the overall phenolic concentration but is strongly influenced by the qualitative composition and structural features of individual compounds. Ethanol-soluble phenolics, particularly flavonoid aglycones and condensed phenolics, are known to exhibit enhanced redox properties and greater efficiency in hydrogen atom or electron donation, thereby contributing to stronger free-radical scavenging activity[40-43]. Structure-activity relationship studies have demonstrated that planarity of the flavonoid nucleus, the presence of ortho-dihydroxyl groups, and the degree of conjugation significantly influence radical scavenging efficacy[44].

The concordant trends observed in both DPPH and ABTS assays further reinforce the reliability of the

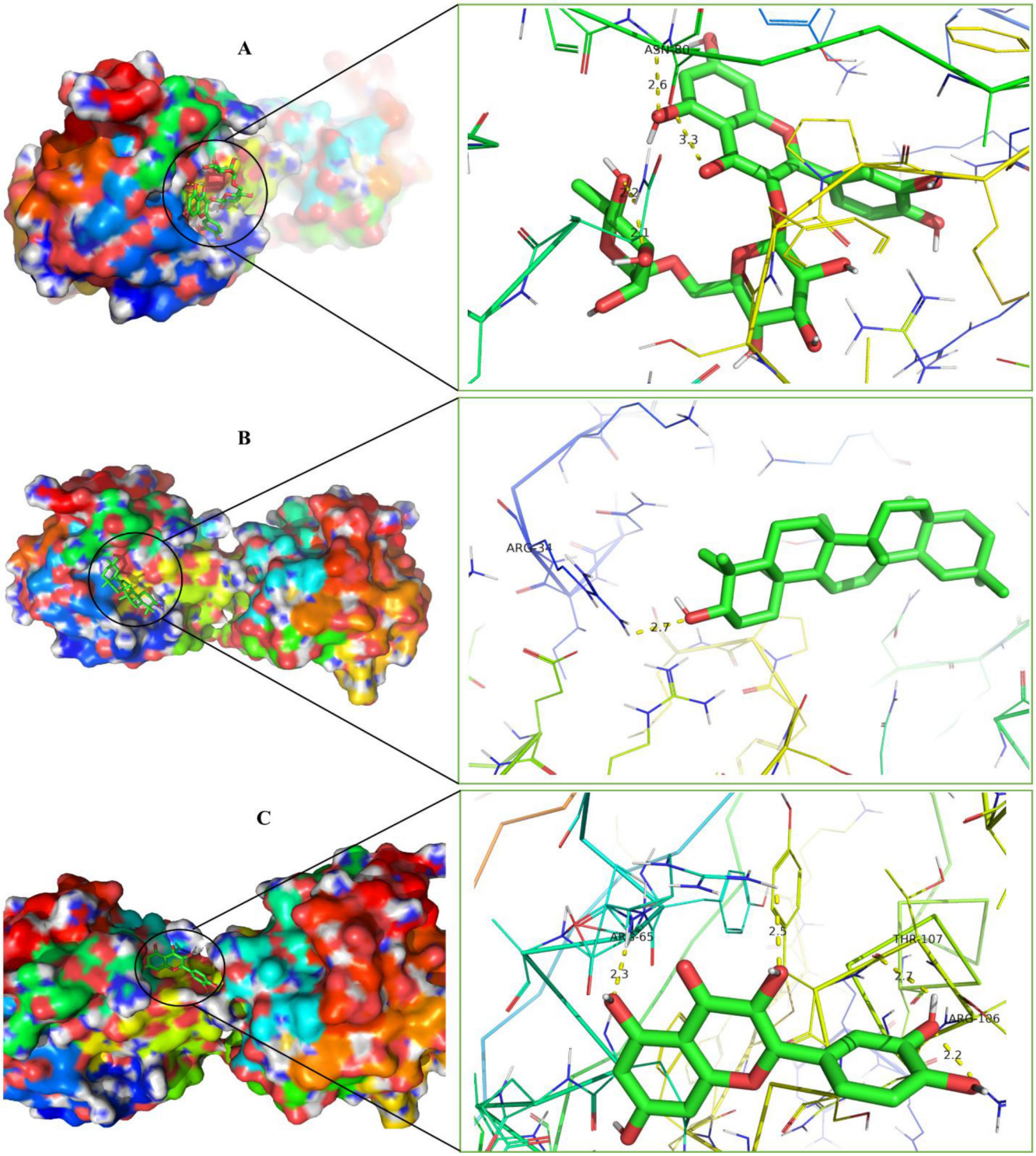


Figure 4. Molecular interactions of phytoconstituents rutin **(A).**, β-amyrin **(B).**, and reference drug quercetin **(C).** with human glutathione peroxidase (PDB ID: 2P31).

antioxidant findings. While the DPPH assay primarily reflects hydrogen-donating ability toward nitrogen-centred radicals, the ABTS assay evaluates scavenging against both hydrophilic and lipophilic radical systems. The consistent performance of the ethanolic extract in both assays indicates a relatively broad-spectrum antioxidant capacity and suggests that the active constituents are capable of neutralising diverse reactive

species[45,46]. Such assay-independent antioxidant responses are generally considered indicative of genuine redox activity rather than method-specific effects. Collectively, the results of this study confirm that *C. sinensis* stems are a rich source of bioactive polyphenols with significant antioxidant potential. The differential solvent responses observed emphasise the importance of extraction strategy in maximising both phenolic yield and biological activity.

Docking analysis confirms that *C. sinensis* stem phytoconstituents—particularly rutin, β-amyrin, floridanine, and flavonoid derivatives—exhibit favourable docking scores and stable interaction patterns comparable to or exceeding the reference quercetin. These findings provide molecular-level support for the experimentally observed antioxidant capacity and validate the contribution of phenolic-flavonoid richness to GPx-associated redox modulation.

4. Limitations and Future Prospects

The present findings provide a biochemical rationale for the traditional use of *C. sinensis* and support further phytochemical characterisation, isolation of active constituents, and pharmacological evaluation to elucidate their therapeutic potential. However, the study lacks *in vivo* validation of the findings derived from the *in vitro* and *in silico* analyses. Future investigations should include molecular dynamics simulations to validate the stability of the docking complexes. In addition, pharmacokinetic and toxicity studies are necessary to evaluate the drug-like properties and safety profile of the identified bioactive compounds.

5. Conclusion

The present study demonstrates that *C. sinensis* Lam. stems are a valuable source of antioxidant phytochemicals with solvent-dependent compositional and functional variation. While aqueous extracts yielded higher total phenolic and flavonoid contents, the ethanolic extract exhibited superior DPPH and ABTS radical scavenging activity, achieving IC_{50} values comparable to quercetin. Molecular docking further supported these findings by revealing stable binding of major stem phytoconstituents to human glutathione peroxidase, suggesting a complementary enzymatic antioxidant mechanism. This integrated experimental and computational study highlights the stem of *C. sinensis* as an underexplored plant resource with significant antioxidant potential, warranting further phytochemical isolation and mechanistic validation.

6. Acknowledgements

The authors sincerely acknowledge the Management and the Dean of the SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur, Tamil Nadu, for their constant encouragement and for providing the necessary infrastructural facilities and support that greatly facilitated the successful completion of this research work.

7. Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

8. References

1. Reddy VP. Oxidative stress in health and disease. Biomedicines. 2023; 11(11):1-17. https://doi.org/10.3390/biomedicines11112925 PMid:38001926 PMCid:PMC10669448
2. Olufunmilayo EO, Gerke-Duncan MB, Holsinger RMD. Oxidative stress and antioxidants in neurodegenerative disorders. Antioxidants. 2023; 12(2):1-30. https://doi.org/10.3390/antiox12020517 PMid:36830075 PMCid:PMC9952099
3. Sun W, Shahrajabian MH. Therapeutic potential of phenolic compounds in medicinal plants-natural health products for human health. Molecules. 2023; 28(4):1845. https://doi.org/10.3390/molecules28041845 PMid:36838831 PMCid:PMC9960276
4. Mei H, Li Y, Wu S, He J. Natural plant polyphenols contribute to the ecological and healthy swine production. J Anim Sci Biotechnol. 2024; 15(1):1-26. https://doi.org/10.1186/s40104-024-01096-3 PMid:39491001 PMCid:PMC11533317
5. Xu L, Wang X. A comprehensive review of phenolic compounds in horticultural plants. Int J Mol Sci. 2025; 26(12):1-25. https://doi.org/10.3390/ijms26125767 PMid:40565226 PMCid:PMC12193087
6. Zagoskina NV, Zubova MY, Nechaeva TL, Kazantseva VV, Goncharuk EA, Katanskaya VM, *et al.* Polyphenols in plants: Structure, biosynthesis, abiotic stress regulation, and practical applications (review). Int J Mol Sci. 2023;

24(18):13874. https://doi.org/10.3390/ijms241813874 PMid:37762177 PMCid:PMC10531498
7. Mueed A, Shibli S, Al-Quwaie DA, Ashkan MF, Alharbi M, Alanazi H, *et al*. Extraction, characterization of polyphenols from certain medicinal plants and evaluation of their antioxidant, antitumor, antidiabetic, antimicrobial properties, and potential use in human nutrition. Front Nutr. 2023; 10(June):1-24. https://doi.org/10.3389/fnut.2023.1125106 PMid:37415912 PMCid:PMC10320526
8. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2009 ;2(5):270-278. https://doi.org/10.4161/oxim.2.5.9498 PMid:20716914 PMCid:PMC2835915
9. Zeukang RD, Kalinski JC, Tembeni B, Goosen ED, Tembu J, Tabopda Kuiate T, *et al*. Quinones from Cordia species from 1972 to 2023: Isolation, structural diversity and pharmacological activities. Nat Prod Bioprospect. 2023; 13(54):1-24. https://doi.org/10.1007/s13659-023-00414-y PMid:37996570 PMCid:PMC10667191
10. Oza MJ, Kulkarni YA. Traditional uses, phytochemistry and pharmacology of the medicinal species of the genus Cordia (Boraginaceae). J Pharm Pharmacol. 2017; 69(7):755-789. https://doi.org/10.1111/jphp.12715 PMid:28266011
11. Matias EFF, Alves EF, Silva MKd, Carvalho VRd, Coutinh HDM, da Costa JGM. The genus Cordia: Botanists, ethno, chemical and pharmacological aspects. Rev Bras Farmacogn. 2015; 25(5):542-552. https://doi.org/10.1016/j.bjp.2015.05.012
12. Rawal P, Jayaseelan K, Ashok P. Standardizing *Cordia sinensis* Lam. stems: Microscopic analysis and TLC fingerprinting of phenolics and flavonoids. Pharmacogn J. 2024; 16(6):1264-1271. https://doi.org/10.5530/pj.2024.16.205
13. Gorgi S, Kiani-Anbouhi R, Doosti R, Asadian M. Extraction of secondary metabolites and computational prediction of their anti-inflammatory potential. RSC Adv. 2025; 15(40):33601-33609. https://doi.org/10.1039/D5RA05230K PMid:40959308 PMCid:PMC12434588
14. Navarrete-Carriola DV, Paz-González AD, Vázquez-Jiménez LK, De Luna-Santillana E, Cruz-Hernández MA, Bandyopadhyay D, *et al*. Comparative analysis of a secondary metabolite profile from roots and leaves of iostephane heterophylla by UPLC-MS and GC-MS. ACS Omega. 2024; 9(5):5429-5439. https://doi.org/10.1021/acsomega.3c06800 PMid:38343925 PMCid:PMC10851369
15. San AMM, Thongpraditchote S, Sithisarn P, Gritsanapan W. Total phenolics and total flavonoids contents and hypnotic effect in mice of *Ziziphus mauritiana* Lam. seed extract. Evidence-based Complement Altern Med. 2013; 2013(May):4-7. https://doi.org/10.1155/2013/835854 PMid:23861716 PMCid:PMC3687505
16. Medini F, Fellah H, Ksouri R, Abdelly C. Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant *Limonium delicatulum*. J Taibah Univ Sci. 2014; 8(3):216-224. https://doi.org/10.1016/j.jtusci.2014.01.003
17. Thapa S, Pandey D, Biradar MS, Arora M, Varghese SA, Elias A, *et al*. Phytochemical profile and α-amylase inhibitory activity of pomegranate peel extract: An *in silico* and *in vitro* investigation. Food Sci Nutr. 2025; 13(11):1-15. https://doi.org/10.1002/fsn3.71191 PMid:41234607 PMCid:PMC12605963
18. Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol. 2011; 48(4):412-422. https://doi.org/10.1007/s13197-011-0251-1 PMid:23572765 PMCid:PMC3551182
19. Sadik S, Vasia, Maiti AK, Shivapooji BA, Jana S. Green synthesis of silver nano particles structural characterization and their antioxidant and anticancer potential using adenocarcinoma (A549) cell line. Indian J Pharm Educ Res. 2024; 58(3):s995-s1007. https://doi.org/10.5530/ijper.58.3s.99
20. Pandey B, Yadav RK, Subedi L, Sapkota B, Baral M, Jha PK, *et al*. Phytochemical investigation and antioxidant activity of *Millettia extensa* against mushroom tyrosinase enzyme: Molecular insight into skin care products. Nat Prod Commun. 2025; 20(1):1-13. https://doi.org/10.1177/1934578X251318518
21. Ulewicz-Magulska B, Wesolowski M. Antioxidant activity of medicinal herbs and spices from plants of the *Lamiaceae*, *Apiaceae* and *Asteraceae* families: Chemometric interpretation of the data. Antioxidants. 2023; 12(12):1-19. https://doi.org/10.3390/antiox12122039 PMid:38136159 PMCid:PMC10740862
22. Vivek-Ananth RP, Mohanraj K, Sahoo AK, Samal A. IMPPAT 2.0: An enhanced and expanded phytochemical atlas of Indian medicinal plants. ACS Omega. 2023; 8(9):8827-8845. https://doi.org/10.1021/acsomega.3c00156 PMid:36910986 PMCid:PMC9996785
23. Al-Musayeib N, Perveen S, Fatima I, Nasir M, Hussain A. Antioxidant, anti-glycation and anti-inflammatory activities of phenolic constituents from *Cordia sinensis*. Molecules. 2011; 16(12):10214-10226. https://doi.org/10.3390/molecules161210214 PMid:22158590 PMCid:PMC6264710
24. Thapa S, Maurya SN, Manjunath K, Mahmood AA, Devi K, Varghese SA, *et al*. LC-MS profiling and multi-target mechanistic insights of *Hibiscus rosa*-sinensis in diabetes: Network pharmacology, molecular docking, MD simulation, PCA, and *in-vitro* α-amylase inhibition. Pharmacol Res - Mod Chinese Med. 2025; 16(May):100636. https://doi.org/10.1016/j.prmcm.2025.100636
25. Prasad S, Kurmi C, Thapa S, Karati D. Molecular docking and pharmacokinetic evaluations of curcumin - based scaffolds as MDM2 - p53 inhibitors. Discov Chem. 2025; 2(53):1-18. https://doi.org/10.1007/s44371-025-00128-9

26. Gonzales AL, Huang SKH, Sevilla UTA, Hsieh CY, Tsai PW. *In silico* analysis of anti-inflammatory and antioxidant properties of bioactive compounds from *Crescentia cujete* L. Molecules. 2023; 28(8):1-14. https://doi.org/10.3390/molecules28083547 PMid:37110781 PMCid:PMC10145697
27. Thapa S, Biradar MS, Banerjee J, Karati D. *In silico* approach for predicting the inhibitory effect of home remedies on severe acute respiratory syndrome coronavirus-2. Makara J Sci. 2023; 27(3):194-207. https://doi.org/10.7454/mss.v27i3.1609
28. Brigelius-Flohé R, Maiorino M. Glutathione peroxidases. Biochim Biophys Acta - Gen Subj. 2013; 1830(5):3289-3303. https://doi.org/10.1016/j.bbagen.2012.11.020 PMid:23201771
29. Margis R, Dunand C, Teixeira FK, Margis-Pinheiro M. Glutathione peroxidase family - An evolutionary overview. FEBS J. 2008; 275(15):3959-3970. https://doi.org/10.1111/j.1742-4658.2008.06542.x PMid:18616466
30. Kurmi SPC, Thapa S, Mahmood AAR, Murav AK, Biradar MS, Bhagyanath N, *et al*. Medicinal chemistry insights of molecular docking and cell permeability mechanism of phytoconstituents present in *Mimosa pudica* as NS2B-NS3 protease inhibitors against dengue virus. Discov Chem. 2025; 2(1):78. https://doi.org/10.1007/s44371-025-00165-4
31. Jendele L, Krivak R, Skoda P, Novotny M, Hoksza D. PrankWeb: A web server for ligand binding site prediction and visualization. Nucleic Acids Res. 2019; 47(W1):W345-W349. https://doi.org/10.1093/nar/gkz424 PMid:31114880 PMCid:PMC6602436
32. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2009; 31(2):455-461. https://doi.org/10.1002/jcc.21334 PMid:19499576 PMCid:PMC3041641
33. Dwivedi MK, Sonter S, Mishra S, Patel DK, Singh PK. Antioxidant, antibacterial activity, and phytochemical characterization of *Carica papaya* flowers. Beni-Suef Univ J Basic Appl Sci. 2020; 9(1):23. https://doi.org/10.1186/s43088-020-00048-w
34. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, *et al*. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. J Food Drug Anal. 2014; 22(3):296-302. https://doi.org/10.1016/j.jfda.2013.11.001 PMid:28911418 PMCid:PMC9354875
35. Khoddami A, Wilkes MA, Roberts TH. Techniques for analysis of plant phenolic compounds. Molecules. 2013; 18(2):2328-23275. https://doi.org/10.3390/molecules18022328 PMid:23429347 PMCid:PMC6270361
36. Chaves JO, de Souza MC, da Silva LC, Lachos-Perez D, Torres-Mayanga PC, Machado AP da F, *et al*. Extraction of flavonoids from natural sources using modern techniques. Front Chem. 2020; 8(September):1-25. https://doi.org/10.3389/fchem.2020.507887 PMid:33102442 PMCid:PMC7546908
37. Zhang H, Hassan YI, Liu R, Mats L, Yang C, Liu C, *et al*. Molecular mechanisms underlying the absorption of aglycone and glycosidic flavonoids in a Caco-2 BBe1 Cell Model. ACS Omega. 2020; 5(19):10782-10793. https://doi.org/10.1021/acsomega.0c00379 PMid:32455198 PMCid:PMC7240828
38. Berga M, Logviss K, Lauberte L, Paulausks A, Mohylyuk V. Flavonoids in the spotlight: Bridging the gap between physicochemical properties and formulation strategies. Pharmaceuticals. 2023; 16(10):1-28. https://doi.org/10.3390/ph16101407 PMid:37895878 PMCid:PMC10610233
39. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. J Nutr Biochem. 2002; 13(10):572-584. https://doi.org/10.1016/S0955-2863(02)00208-5 PMid:12550068
40. Pietta PG. Flavonoids as antioxidants. J Nat Prod. 2000; 63(7):1035-1042. https://doi.org/10.1021/np9904509 PMid:10924197
41. Foti MC. Antioxidant properties of phenols. J Pharm Pharmacol. 2007; 59(12):1673-1685. https://doi.org/10.1211/jpp.59.12.0010 PMid:18053330
42. Shahidi F, Ambigaipalan P. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects - A review. J Funct Foods. 2015; 18(July):820-897. https://doi.org/10.1016/j.jff.2015.06.018 PMCid:PMC11340972
43. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med. 1996; 20(7):933-956. https://doi.org/10.1016/0891-5849(95)02227-9 PMid:8743980
44. Seyoum A, Asres K, El-Fiky FK. Structure-radical scavenging activity relationships of flavonoids. Phytochemistry. 2006; 67(18):2058-2070. https://doi.org/10.1016/j.phytochem.2006.07.002 PMid:16919302
45. Platzer M, Kiese S, Herfellner T, Schweiggert-Weisz U, Miesbauer O, Eisner P. Common trends and differences in antioxidant activity analysis of phenolic substances using single electron transfer based assays. Molecules. 2021; 26(5):1-17. https://doi.org/10.3390/molecules26051244 PMid:33669139 PMCid:PMC7956415
46. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem. 2005; 53(10):4290-4302. https://doi.org/10.1021/jf0502698 PMid:15884874

Author Contribution

Pinkey Rawal: Conceptualisation, methodology, data curation, formal analysis, writing - original draft. Kavitha Jayaseelan: Conceptualisation, formal analysis, writing - review and editing, supervision. Purnima Ashok: Data analysis, writing - review and editing. All authors have read and agreed to the published version of the manuscript.