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Research Article

Efficacy of *Carica papaya* Leaves Extract for Treating Thrombocytopenia: An *In Silico* and *In Vivo* Study in Rat Model

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Abstract | The aim of this study was to determine the anti-thrombocytopenic activity of the ethanol extract of *C. papaya* leaves in rats using *in silico* and *in vivo* studies. Analysis of phytochemical compounds was carried out using GCMS and qualitative phytochemical screening. Additionally, antioxidant activity was determined by spectrophotometry. A rat model of thrombocytopenia was established by subcutaneous injection of heparin at 2000 IU/kg body weight (BW) for 10 days. After induction, rats were administered *C. papaya* leaves ethanol extract at various concentrations (50, 100, and 200 mg/kg BW) for 20 days. On day 21, the duke method was used to examine the bleeding time, and the blood clotting time was evaluated using the Lee White method. Blood samples were evaluated for platelet concentration, thrombopoietin concentration, prothrombin time, and active partial thromboplastin time. These results indicated that the C. papaya leaf extract contained various compounds. ABTS antioxidant activity assay showed an IC_{50} of 45.50 µg/ml. *In vivo* examination revealed that C. papaya extract has potential anti-thrombocytopenic effects; doses of 100 and 200 mg/kg BW showed significant improvement in the hematology profile from that of the heparin control ($p < 0.05$). In particular, doses of 200 mg/kg BW have the best potential for the development of anti-thrombocytopenic agents. Furthermore, an *in silico* study found that the main compound of *C. papaya* interacts with multiple amino acid residues, indicating stable binding affinity. Finally, it can be concluded that *C. papaya* has potential as an anti-thrombocytopenic agent.

Keywords | *Carica papaya*, Hematology, Heparin, *in silico*, *in vivo*, Thrombocytopenia

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INTRODUCTION

Thrombocytopenia is a hematological ailment characterized by a reduction in the quantity of platelets in the blood vessels. This condition poses substantial therapeutic challenges because thrombocytes have a vital function in the process of blood clotting, and a lack of platelets can result in bleeding disorders (Singh *et al.*,

2021). This ailment can either be idiopathic or occur as a result of several conditions such as leukemia, anemia, viral infections (including dengue fever), and chemotherapy (Hamamyh and Yassin, 2019). Typical approaches to managing thrombocytopenia include platelet transfusions, corticosteroids, and immunoglobulin therapy (Gafter-Gvili, 2023). Nevertheless, these therapies are not exempt from limitations and hazards such as the possibility of immunological reactions, restricted availability of donors, and varying responses from patients. Considering these circumstances, investigation into alternative therapies is not just pertinent, but imperative. Herbal medicine, known for its extensive historical background and limited adverse reactions, presents a highly encouraging path. *C. papaya* leaf extract is a promising herbal remedy for the treatment of thrombocytopenia. *C. papaya* is a tropical fruit renowned for its nutritional and therapeutic properties. Papaya leaves have been utilized in traditional medicine across diverse cultures to treat a variety of diseases.

Recent pharmacological studies indicate that *C. papaya* leaf extract can enhance platelet formation, making it a viable treatment drug for thrombocytopenia (Nandini *et al*., 2021). Multiple clinical trials and observational studies have demonstrated that administering *C. papaya* leaf extract to patients, especially those with thrombocytopenia caused by dengue fever, results in a rapid elevation in platelet counts (Sarker *et al*., 2021; Shrivastava *et al*., 2022). Additionally, Other studies have demonstrated the bioactive components of *C. papaya*, such as carpaine and quercetin, have been identified as key contributors to this effect (Munir, 2022; Nandini *et al*., 2021). Nevertheless, scientific investigation of *C. papaya* leaf extract in relation to thrombocytopenia has primarily focused on observational pre-clinical results and the precise biochemical and molecular mechanisms responsible for the hematological effects of *C. papaya* leaf extract have not been comprehensively characterized.

This lack of comprehension represents a substantial deficiency in research. Empirical evidence supports the effectiveness of *C. papaya* leaf extract in increasing platelet count. However, the lack of comprehensive knowledge of its mechanism of action hinders its acceptance and integration into conventional medical practice. This is of major significance because of the complex characteristics of the hematological system and numerous factors, including thrombopoietin, cytokines, and other growth factors that regulate platelet formation and function (Boscher *et al*., 2020).

To fill this gap, we examine the effectiveness of *C. papaya* leaf extract in treating thrombocytopenia. A comprehensive strategy that combines computer-based simulations (*in silico*) with experiments on living organisms (*in vivo*) were conducted. The *in silico* component uses computational analysis and simulation tools to propose how the phytochemicals in *C. papaya* leaf extract interact with biological pathways pertinent to platelet synthesis and function. Utilizing this method is essential for recognizing possible molecular targets and understanding the bioactivity profile of the phytochemical components of *C. papaya* leaves. Conversely, the *in vivo* component of our investigation will offer empirical evidence to verify the assumptions derived from in silico analysis. This will entail performing controlled tests on animal models to directly observe the effects of *C. papaya* leaf extract platelet count and other hematological parameters. Conducting these tests is crucial for determining the safety, effectiveness, and optimal dosage of *C. papaya* leaf extract in a biological system. This will provide the necessary foundation for future clinical trials. It hypothesizes that *C. papaya* leaf extract can significantly improve platelet counts. By integrating computational predictions with biological evidence from rat models, the research aims to validate the extract's effectiveness and uncover the mechanisms behind its therapeutic action, paving the way for its application in human treatments for thrombocytopenia.

MATERIALS AND METHODS

Materials

The materials used were 96% ethanol (Merck), ethyl acetate (Merck), chloroform (Merck), heparin (inviclot), ELISA Kit Trombopoetin (E-Lab Science), quercetin (Sigma), ABTS (Sigma), potassium persulfate (Sigma), and distilled water. The tools used were laboratory glassware, microtitration plate 96, microtube, rotary evaporator (Mingyi), Coatron® A4 Fully Automated Hemostasis Analyzer, Hematology Analyzer XN 450 (Sysmex), UV-Vis Spectrophotometer (Peak), and an ELISA microplate reader (DIATEK). The study utilized thirty-five male Wistar rats, aged 10-12 weeks, with a weight ranging from 150 to 180 grams. The rats and pellet food were acquired from the Rat Breeding Centre, Pharmacology Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara. The rats were kept in a controlled environment with a consistent temperature and humidity, and a 12-hour light and dark cycle. The animals were provided with a standardized laboratory pellet diet and tap water for feeding. The animals undergoing testing were acclimatized for a duration of one week before the investigation commenced. The blood sample was obtained using a heart puncture. The project has received clearance from the Animal Research Ethics Committees (AREC) of Universitas Sumatera Utara, with the assigned approval number 0345/KEPH-FMIPA/2023.

Plant collection and extract preparation

C. papaya leaf samples were collected form Ganjos papaya

farming at Binjai City, North sumatera, Indonesia. Clean *C. papaya* leaf were cut uniformly, then dry them in a cabinet set to 50°C. Space them evenly for optimal airflow and monitor to prevent over or under-drying. After drying, store them in airtight containers in a cool, dark place to maintain their medicinal properties.

Powdered dried leaves (300 g) were extracted by maceration with a mixture of ethanol and water (70:30, v/v). The extraction process required continuous agitation at 25 °C. After 24 h, the mixture was filtered off. The procedure was duplicated on 2 separate occasions, leading to a cumulative count of the extractions. The collected samples were combined and centrifuged at 3500 rpm for 10 min at room temperature. The liquid was condensed using a rotary evaporator at 38°C, resulting in the production of the hydroethanolic extract (HESc). The HESc sample was subjected to three rounds of separation using chloroform (1:1 v/v), followed by three rounds of extraction of the aqueous phase using ethyl acetate (1:1 v/v). The ethyl acetate fraction was subjected to evaporation under low pressure and subsequent freeze-drying, leading to the formation of a polyphenol-enriched extract (PESc) (Chagas *et al.*, 2018).

Phytochemical constituent analysis

GC-MS was used to determine the phytochemical content (Marianne *et al.*, 2021). In addition, the presence of various phytochemicals, including alkaloids, flavonoids, glycosides, tannins, saponins, triterpenoids, and steroids, was examined employing established qualitative protocols. The detection of flavonoids was conducted using a reagent consisting of hydrochloric acid (concentrated), magnesium powder, and amyl alcohol. Alkaloids were identified using Mayer's, Bouchardat's, and Dragendorff's reagents, respectively. The foam test was applied for the identification of saponins. Tannins were detected with the aid of the ferric chloride reagent, and the presence of steroids and terpenoids was determined using the Liebermann-Burchard reagent (Banu and Cathrine, 2015).

Determination of ABTS radical scavenging activity

ABTS radical cation decolorization assay was used to determine the ability of the plant materials to remove free radicals. This process was reproduced as previously described, using quercetin as the positive control (Gülçin, 2010).

Treatment design

Rats were divided into five groups: group 1 (normal group), Group 2 (heparin group); and groups 3, 4, and 5 as extract treatment groups with doses of 50, 100, and 200 mg/kg BW, respectively. Thrombocytopenia was induced in a rat model via subcutaneous injection of heparin (Inviclot) at a dosage of 2000 IU/kg body weight (BW) for 10 days (Zahroh *et al.*, 2016). Following verification of platelet count reduction, the *C. papaya* leaf ethanol extract was orally administered to rodents for a duration of 20 days. The bleeding time was assessed on day 21 following the final administration of the extract. Additionally, the hematological profile was determined using the Hematology Analyzer XN 450, thrombopoietin levels were determined using an ELISA microplate reader (González-González *et al.*, 2021), and the prothrombin time and active partial thromboplastin time were evaluated using the Coatron® A4 Fully Automated Hemostasis Analyzer (Kochhar and Neunert, 2021).

In silico tools

The equipment consisted of an HP Laptop with a Windows 11 operating system, 64-bit architecture, 4 GB RAM, 256 GB SSD, and a 14-inch display. This study employs various software tools for different purposes. These included the Windows 11 64-bit operating system, Chimera 1.16, for visualizing molecular structures, the Protein Data Bank for accessing protein structure data, PubChem for accessing information on chemical compounds, and Swiss Dock for conducting protein-ligand docking simulations.

Preparation of ligands and proteins

The thrombopoietin receptor gene (TpoR) was obtained from the Protein Data Bank website (*). PDB file format. Subsequently, the UCSF Chimera 1.16 tool was used to prepare the sample by eliminating residues. The test compounds were generated using UCSF Chimera 1.16. This was achieved by inputting the PubChem CID of the ligand, which was acquired earlier using the PubChem online service and stored in the mol2 format. Molecular docking involves interactions between proteins and either test chemicals or natural ligands, and the Swiss Dock platform was used to execute the docking procedure. For molecular docking to function with Swiss Dock, the target protein and ligand files must be meticulously prepared, including protonation, optimization, and verification of correct 3D conformations. The interface of Swiss Dock enables users to customize parameters such as ligand flexibility and the docking approach (blind or focussed) in order to align with particular research objectives. Furthermore, redocking between the natural ligand and the protein was also performed to validate the method.

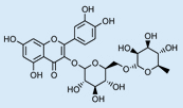
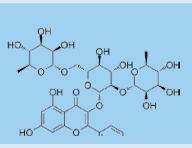
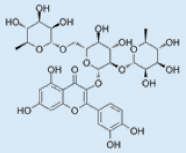
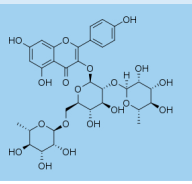
Docking data were quantified using the Gibbs free energy (ΔG) value (Martins da Silva *et al.*, 2023). Table 1 lists the precise attributes of these ligands.

Rendering of docking outcomes

The visualization process was performed using the USCF Chimera 1.16. Protein data and docking results were

entered into*. pdb file format. Visualization illustrates the specific type of bond interaction established together with the amino acid that serves as the binding site. The visualization results are presented in *. png file format (Pettersen *et al.*, 2004).

Table 1: Ligand name.

Name	Formula	Chemical structure
Rutin	C27H30O16	
Carpaine	C28H50N2O4	
Manghaslin	C33H40O20	
Clitorin	C33H40O19	

Statistical analysis

The *in vivo* results were analyzed using ANOVA with Tukey's multiple comparison test. P-values for significance were set at $P<0.05$. Values for all measurements are expressed as mean±SD. The histogram data were constructed using GraphPad Prism Software 9.0.

RESULTS AND DISCUSSION

Phytochemical constituent and antioxidant analysis

Qualitative analysis of the compounds in the extracts of papaya leaves was performed using the standard procedure shown in Tables 2, 3 and Figure 1.

Table 2: Phytochemical screening result.

No.	Content	Reagent	Dried sample	Polyphenol rich extract
1	flavonoids	HCL(c), Mg powder. Amyl alcohol	+	+
2	alkaloids	Mayer	+	-
		Bouchardat	+	-
		Dragendorf	+	-
3	saponins	Foam test	+	+
4	tannins	FeCl3	+	+
6	steroids/ terpenoid	Liberman Burchard	+	-

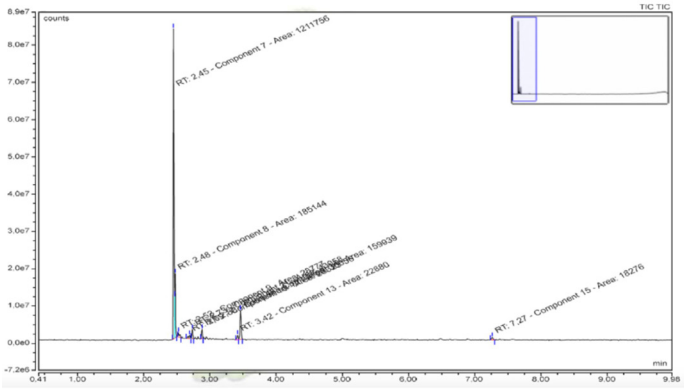


Figure 1: Gas chromatography/mass spectrometry (GC/MS) chromatograms

The phytochemical screening results indicated that the leaf extracts of *C. papaya* leaves contained a diverse range of chemicals. Both the dehydrated sample and the extract abundant in polyphenols contained flavonoids, saponins, tannins, and various other chemicals, suggesting a wide-ranging phytochemical composition. The specific extraction process used to enrich the polyphenol content may result in the potential omission of alkaloids and steroids/terpenoids from the polyphenol-rich extract, unlike in the desiccated sample. Numerous studies have identified a variety of phytochemicals, including alkaloids, flavonoids, saponins, tannins, and glycosides, in papaya leaves, which supports this finding (Ikeyi *et al.*, 2013; Nath and Dutta, 2016).

Table 3: GCMS phytochemical analysis result.

No	Chemical name	Molecular weight (g/mol)	Molecular formula	Retention time (Min)	Relative area (%)
1	Oxalyl chloride	126.92	C2Cl2O2	2.45	69.77
2	Acetyl chloride	78.50	C2H3ClO	2.48	10.66
3	N, N-Bis (2-hydroxyethyl)-2-aminoethanesulfonic acid	213.25	C6H15NO5S	2.52	1.66
4	Oxirane, 2,3-dimethyl-, trans-	72.11	C4H8O	2.69	1.63
5	sec-Butyl nitrite	103.12	C4H9NO2	2.73	2.48
6	Thiocyanic acid, methyl ester	73.12	C2H3NS	2.88	2.22
7	3,6,9,12-Tetraoxahexadecan-1-ol	250.33	C12H26O5	3.42	1.32
8	1,3,5-Cycloheptatriene	92.14	C7H8	3.46	9.21
9	Oxacyclododecan-2-one	184.27	C11H20O2	7.27	1.05

Additionally, The GCMS chromatogram in Figure 1 and compound data in Table 3 provide a detailed description of the compounds identified. Oxalyl chloride was the most abundant compound, occupying 69.77% of the relative area with a retention time of 2.45 minutes. Furthermore, the presence of acetyl chloride and a range of organic compounds with various functional groups, such as N, N-bis (2-hydroxyethyl)-2-aminoethanesulfonic acid and 1,3,5-Cycloheptatriene, indicate a complex phytochemical profile that may contribute to the biological activities of papaya leaves.

Table 4: ABTS antioxidant assay result.

Sample	Concentration (ppm)	% Inhibition	IC_{50} value (µg/ml)
C. papaya leaves extract	10	48.32	45.50
	20	52.45	
	40	59.67	
	60	67.46	
	80	71.85	

Table 4 shows the IC_{50} value is a significant indicator of the potency of the antioxidant property of a substance, and represents the concentration required to inhibit 50% of the radical cation ABTS·+. An IC_{50} value of 45.50 µg/ml for papaya leaf extract is indicative of a potent antioxidant effect, as lower IC_{50} values correspond to higher antioxidant activity. These findings suggest that *C. papaya* leaf extract possesses substantial free radical scavenging activity, which may contribute to its potential therapeutic benefits. Antioxidants play a crucial role in hematopoietic cell function. They protect hematopoietic stem cells from oxidative stress, which helps to maintain their capacity to regenerate and transform into different types of blood cells (Chen *et al*., 2020). This protection is particularly important for the maturation of blood cells, as antioxidants aid in maintaining the equilibrium required for hematopoiesis (Nisha and Deshwal, 2011). They also safeguard against DNA damage and improve the survival of hematopoietic cells, as demonstrated in animal studies (Wambi *et al*., 2008).

Anti-thrombocytopenic evaluation

Statistical analyses for anti-thrombocytopenic evaluation were conducted using ANOVA, with preliminary tests to check for normal distribution (Shapiro-Wilk test, $p > 0.05$) and homogeneity of variances (Levene's test, $p > 0.05$). Post-hoc comparisons were made using Tukey's HSD test, and for multiple comparisons, we applied the Dunnett correction to maintain the overall alpha at 0.05.

Figure 2A and 2C illustrate that the platelet count and thrombopoietin levels in rats treated with 200 mg/kg body weight *C. papaya* extract differed significantly from those in the heparin control group ($P \leq 0.05$), indicating the potential of the extract to mitigate the effects of heparin. Conversely, Figure 2B shows a significant reduction in bleeding time across all treatment groups. This anti-thrombocytopenic effect is associated with the constituents of *C. papaya* leaf extract, which contains various phytochemical compounds. *C. papaya* leaf contains various minerals, such as calcium (Ugo *et al*., 2019), which can accelerate the production of thrombin and promote the formation of fibrin threads. This facilitates faster blood clotting, leading to reduced bleeding (Bhattacharjee and Bhattacharyya, 2014). Calcium also contributes to the process of blood clotting. The conversion of factor X to factor Xa and the conversion of prothrombin to thrombin are both reliant on calcium ions, which have vital functions in the coagulation cascade (Camire, 2021). Fukuda *et al*. (2006) observed that rats with hypocalcemia exhibited considerably prolonged bleeding times compared to normal rats, indicating the important function of calcium in the hemostasis process.

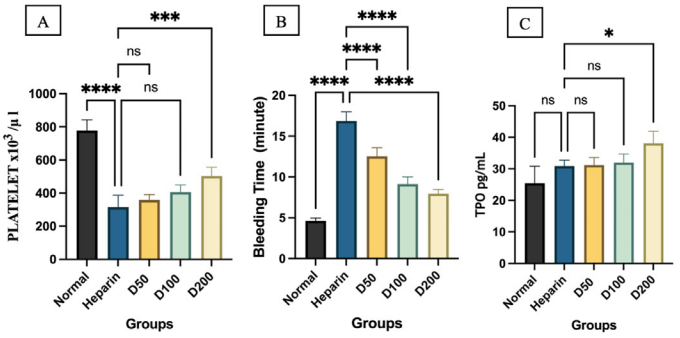


Figure 2: (A) Platelet concentration; (B) Bleeding Time; (C) Thrombopoietin concentration . Asterisk *P ≤ 0.05, **P ≤ 0.01, *** P ≤ 0.001, ****P ≤ 0.0001 represents significant differences to heparin group and NS represents not significant (P > 0.05).

Thrombopoietin is the principal regulator of platelet production; hence, disorders of the hormone or its receptor may also cause thrombocytopenia. Although the thrombopoietin concentration did not vary in the groups treated with 50, 75, and 100 mg/kg BW *C. papaya* extract compared with that of the control group ($P > 0.05$), a significantly higher concentration was found in the group treated with 125 mg/kg BW compared to that in the heparin group (Figure 1C), suggesting that only the high dose influenced thrombopoietin production.

Furthermore, Figure 3 shows that *C. papaya* leaves extract at a dose of 100 and 200 mg/kg BW resulted in an improvement in the prothrombin time and active partial thromboplastin time ($P \leq 0.05$) compared with those of the heparin group (Figure 3A, B). Active partial thromboplastin time is used to evaluate the intrinsic coagulation pathway coagulation factors VIII, IX, XI, and

XII, whereas prothrombin time is used to examine the extrinsic coagulation pathway factors V, VII, and X. The reduction in active partial thromboplastin time following administration of *C. papaya* extract has an influence on the intrinsic coagulation pathway. To our knowledge, no study has reported the coagulant action of *C. papaya* leaves.

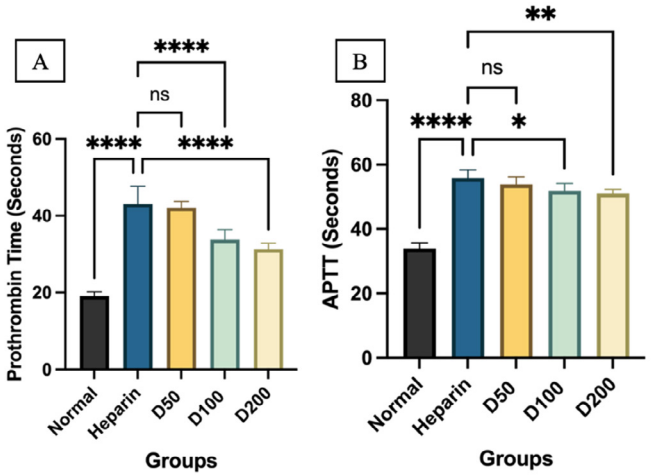


Figure 3: (A) Prothrombin time; (B) APTT. Asterisk *P ≤ 0.05, **P ≤ 0.01, *** P ≤ 0.001, ****P ≤ 0.0001 represents significant differences to heparin group and NS represents not significant (P > 0.05).

Although there is limited research on the potential of *C. papaya* as an anti-thrombocytopenic agent, several preclinical studies have investigated the potential of *C. papaya* as a hematopoietic agent to improve the hematological profile. A study revealed that *C. papaya* juice and fraction intake in rats resulted in an improvement in platelet count (Nandini *et al*., 2021). Another study found that *C. papaya* extract administered at a dose of 200 mg/kg BW significantly improved the hematological profile and increased the platelet count; however, this study was not based on a thrombocytopenic rat model, but rather used a normal animal model (Patil *et al*., 2013). The results of the current study demonstrate a more potent effect at a dose of 200 mg/kg BW) of *C. papaya* leaves extract, which improved the blood profile of rats with thrombocytopenia. Several human studies have demonstrated the positive impact of *C. papaya* juice on hematological parameters. One study found that juice significantly increased platelet and red blood cell counts (Subenthiran *et al*, 2013). This finding is further supported by Yunita *et al*. (2017). However, efficacy and safety investigations are required before *C. papaya* extract can be used in humans.

The phytochemical content of *C. papaya* may play a role in its anti-thrombocytopenic properties. Phenolic group groups in *C. papaya* have been shown to have antioxidant and anti-inflammatory properties, thereby protecting against oxidative stress (Kong *et al*, 2021), which is involved in the development of thrombocytopenia. In 2020, Boojar stated that flavonoids could reduce the rate of cell death of hematopoietic stem cells from 43% to 77% and decrease the suppression of pro-inflammatory factors such as interleukin-6 and cyclooxygenase-2 in mouse bone marrow and spleen cells (Boojar, 2020). Therefore, the flavonoids identified in the *C. papaya* extract may play a role in its anti-thrombocytopenic activity.

Additionaly, Carica papaya leaf extract has been shown to enhance platelet counts in both healthy individuals and those with thrombocytopenia, potentially due to its ability to increase the expression of the CD110 receptor on megakaryocytes (Kurian and DholeNimai,2018; Nandini *et al*.,2021). This extract has also been found to increase platelet count and decrease clotting time in rats (Kad and Tambe, 2018). The presence of bioactive compounds in the extract, such as alkaloids, saponins, and flavonoids, may contribute to its platelet production and differentiation capabilities (Adarsh *et al*, 2023). Furthermore, the extract has been shown to directly inhibit platelet aggregation, particularly during dengue viral infection (Chinnappan *et al*., 2016).

In silico STUDY

The thrombopoietin receptor (TpoR), encoded by the MPL gene, plays a key role in thrombopoietin signaling and regulates megakaryocytopoiesis and platelet formation (Hitchcock *et al*., 2021). Thrombopoietin, the primary regulator of platelet production, binds to the MPL receptor, leading to the activation of JAK2 and TYK2 tyrosine kinases and subsequent signaling (Dib *et al*., 2020). Thrombopoietin and its receptor also play a role in normal and neoplastic hematopoiesis (Kaushansky, 2016). Studies in c-mpl-deficient mice have further demonstrated the specific role of this receptor in regulating platelet production (Gurney and De Sauvage, 1996). Disruptions in TpoR function can lead to various blood disorders. In this study, an analysis model is developed to determine the possible activity of *C. papaya* leaves as an anti-thrombocytopenic agent through *in silico* analysis. The docking results are presented in Table 5 and Figure 4.

The primary goal of our study was to explore the binding interactions of four principal compounds found in *C. papaya* with thrombopoietin receptor (TpoR). Carpaine demonstrates the highest binding affinity (-9.3 kcal/mol), potentially due to its interaction with distinctive residues as well as prevalent ones (e.g., TYR69, GLU72). As a result of these additional contacts, carpaines may induce conformational changes in TPOR or stabilize the receptor in a specific activation state. Moreover, rutin and clitorin exhibited binding patterns comparable to each other and to carpaine, interacting with a core set of amino acids, as evidenced by their respective affinities of -8.7 and -8.9 kcal/mol. This implies the presence of a potentially shared mechanism of action among these compounds,

Table 5: Docking affinity scores.

Ligand	Protein	ΔG (kkal/ mol)	Amino acid residue
Rutin	TPOR	-8.7	LEU62, PRO63, ALA64, VAL65, ASP66, ARG138, THR140, ALA141, HIS142, ASP144, LEU150, SER151, HIS154, PRO70, ARG71, PHE104, PHE105, PRO106, PHE126
Carpaine	TPOR	-9.3	LEU62, PRO63, ALA64, VAL65, ASP66, THR140, ALA141, HIS142, ASP144, ALA147, LEU150, SER151, HIS154, ALA68, TYR69, PRO70, ARG71, GLU72, PHE104, PHE105, PRO106, LEU107, HIS108, VAL124, PHE126
Manghas-lin	TPOR	-8.0	LYS35, ASP39, VAL42, LEU43, ARG46, ARG99, SER108, SER109, GLY112, GLN113, SER115, GLY116, GLN117, LEU120, THR44, GLU46, ASP47, ASP97, GLN98, GLU99
Clitorin	TPOR	-8.9	LEU62, PRO63, ALA64, VAL65, ASP66, ARG138, THR140, ALA141, HIS142, LYS143, ASP144, ASN146, ALA147, LEU150, SER151, HIS154, ALA68, PRO70, ARG71, PHE104, PHE105, PRO106, LEU107, HIS108, VAL124

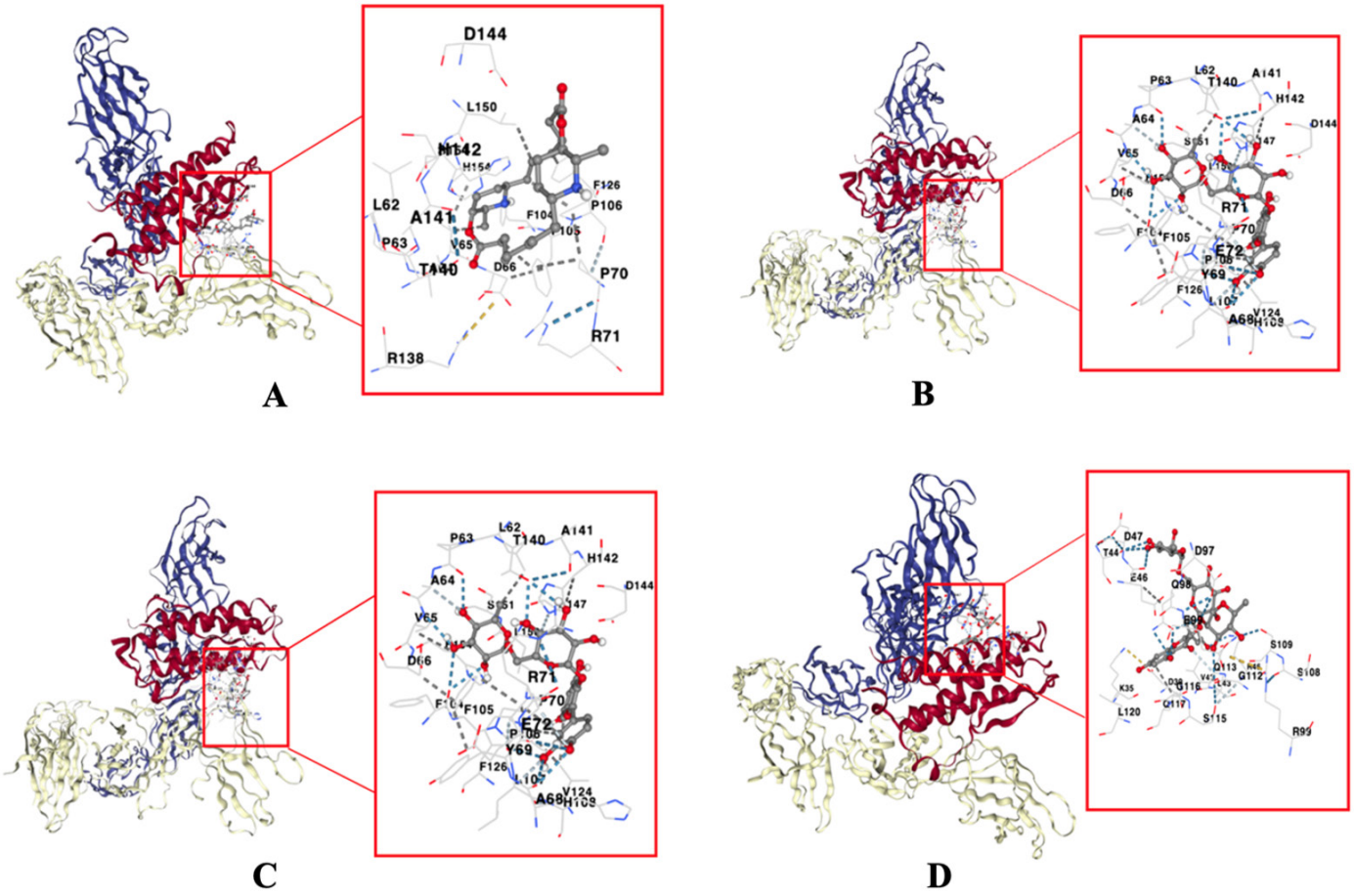


Figure 4: Docking visualization. A, Rutin-TpoR; B, Carpaine -TpoR; C, Manghaslin-TpoR; D, Clitorin- TpoR.

which merits additional investigation to understand the biological implications of these interactions. Additionally, manghaslin interacted with a distinct set of amino acids, despite having the lowest binding affinity (-8.0 kcal/ mol). The distinctive binding profile observed implies that manghaslin may exert distinct effects on TPOR activity, a property that could prove advantageous in therapeutic scenarios involving the manipulation of receptor states.

In contrast, the binding site of TPO on TpoR, more precisely located in residues 206-251 of Mpl-EC domain 1, comprises crucial amino acids, including Leu (228), Leu (230), Asp (235), and Leu (239) (Chen *et al*., 2010). Potential therapeutic implications may result from the way in which each ligand modulates TpoR activity in response to distinct binding locations; this may involve the regulation of megakaryocyte proliferation and platelet formation (Neu *et al*., 2020). The identification of an increased variety of binding sites implies that phytochemicals may be capable of establishing stable interactions with TpoR across a wider range of interaction patterns.

Thus, there is potential for the development of novel Tpo-R-targeting pharmaceuticals with improved

specificity and efficacy. Additionally, a study examining the interactions of mimic feline thrombopoietin, a feline protein resembling human thrombopoietin, concentrated on three specific amino acid sites: Thr 213; Ala 211; and Arg 212 (Matsushiro *et al*., 1998). Furthermore, the current investigation underscores the similar binding sites found in both feline thrombopoietin and several compounds of *C. papaya*, thus highlighting a substantial correlation between their biochemical structures and functions. Further research is necessary to analyze the pharmacodynamic effects and therapeutic ramifications of these innovative binding interactions on *C. papaya* compounds and analogous drugs, particularly those that stimulate thrombopoiesis.

In accordance with in silico docking results, TPO serum concentrations must be correlated with in silico docking affinities to determine *C. papaya* leaf chemicals effects on the thrombopoietin signalling axis. the relationship between in silico binding affinities and serum TPO levels is complex and may be affected by physiological factors like platelet production and destruction, TpoR turnover, and thrombopoiesis regulation. Comprehensive studies using *in vitro* and *in vivo* assays should be conducted to confirm the therapeutic potential of *C. papaya* leaf extracts and the biological relevance of our *in silico* findings.

CONCLUSIONS AND RECOMMENDATIONS

The in silico study suggests that the main compound in *C. papaya* extract binds to TPOr, aiding in the maturation of platelet cells. In animal tests, *C. papaya* leaf extract showed potential as a treatment for thrombocytopenia, with certain doses significantly enhancing blood profiles compared to heparin ($p<0.05$), particularly at 200 mg/kg body weight. While these results are promising, further research, including human trials, is crucial to confirm C. papaya leaf extract's effectiveness against thrombocytopenia in humans. It's also important to fully assess potential side effects and determine safe, effective dosages for clinical use. Thus, these findings should be seen as an early step towards broader research, not as conclusive proof of its clinical value.

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NOVELTY STATEMENT

The novelty of this study is the evaluation of the effect of *C. papaya* leaf extract on the treatment of thrombocytopenia.

AUTHOR'S CONTRIBUTION

All authors contributed equally to the manuscript.

Conflict of interest

The authors declare that they have no conflicts of interest in relation to this research, whether financial, personal, authorship, or otherwise, which could affect the research and the results presented in this paper.

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