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Exploring the potential of *Beta Vulgaris L.* Against thrombocytopenia: *in vitro, in silico,* phytochemicals, and antioxidant analysis

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ABSTRACT

The purpose of this study was to assess anti- thrombocytopenia of beetroot extract using both in vitro and in silico methods. Additionally, antioxidant capacity, phenolic content, and flavonoid content of beetroot were determined. The extraction procedure was optimized by reflux method, the extracts obtained were examined to determine the Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) using the Folin-Ciocalteu colorimetric and aluminum chloride methods, respectively. Furthermore, antioxidant capacity was assessed using the DPPH method, while betanin concentration was determined by thin-layer chromatography. This research approach encompasses the binding acticity of ligands and target proteins, the process of docking thrombopoietin receptor (TPOr) and betanin compound using Swissdock, and the examination of docking data using the USCF Chimera software. The studies revealed that the reflux-extracted beetroot extract had a total phenol concentration of 39.72 ± 2.56 mgGAE/g. The flavonoid concentration of beetroot extract obtained using reflux extraction was 5.47 ± 0.39 mg QE/g.

TLC

Beta vulgaris L.

Phenol, flavonoid and Antioxidant

Phenol: 39.72 ± 2.56 mg GAE/g
Flavonoid: 5.47 ± 0.39 mg QE/g
Antioxidant (IC50): 95.71 µg/mL

Reflux extraction

SwissDock

Thrombopoietin-receptor agonists (TpoR)

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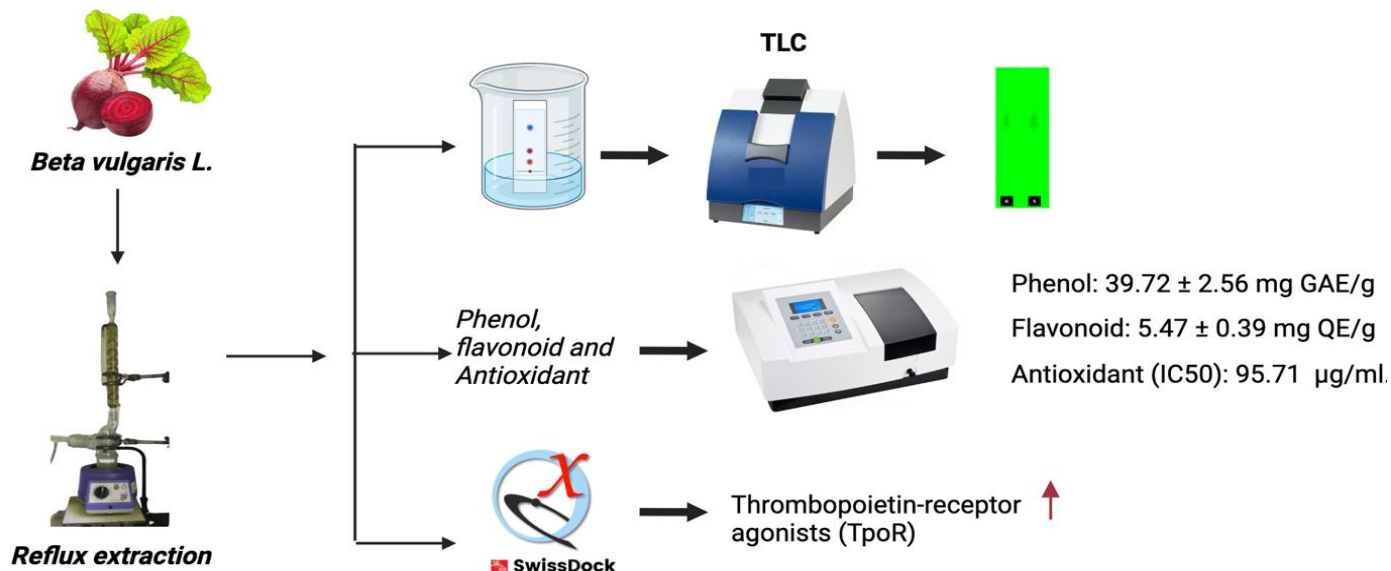
The qualitative test conducted using thin layer chromatography yielded data indicating that the reflux-extracted extract tested positive for betanin. The reflux approach yielded an extract with antioxidant activity (IC50) of 95.71 μg/ml. The results of the molecular docking simulation showed that the betanin compound had a ΔG value of -8.1 Kcal/mol. It interacts with multiple amino acid residues, including LEU62, PRO63, ALA64, VAL65, ASP66, ARG138, THR139, THR140, ALA141, HIS142, ALA147, ILE148, LEU150, SER151, HIS154, PRO70, ARG71, ARG102, PHE104, PHE105, PRO106, and PHE126. An in vitro study demonstrated that the extract of *Beta vulgaris* L. has the ability to induce blood coagulation, indicating its potential as a haemostatic agent. Additionally, in silico investigations suggest that the betanin chemicals found in beetroot serve as TpoR agonists, and it can be concluded that betanin has antithrombocytopenia potential.

Keywords: Antioxidant, Phenolic, Flavonoids, Betanin, Thrombocytopenia.

INTRODUCTION

Thrombocytopenia is a medical condition characterized by a large decrease in the number of platelets, which can lead to serious health risks including severe bleeding and impaired blood clotting [1]. This syndrome can have various causes, such as disorders that damage the bone marrow, specific infections, or negative reactions to certain drugs [2]. Several essential factors contribute to the need to explore alternative therapies such as medicinal plants. First, it is important to acknowledge that while traditional treatments have been proven to be effective, they may not be suitable for every patient owing to possible negative effects or contraindications. Moreover, a significant number of people are unable to access essential medical therapy because of exorbitant expenses and restricted availability of conventional medical remedies [3]. Moreover, historical and empirical evidence substantiates the idea that certain botanicals can augment platelet counts and foster general blood well-being. Exploring these natural therapies could lead to important discoveries and broaden options for treating thrombocytopenia. Nevertheless, caution should be exercised when contemplating alternative medicines of this kind, which should be substantiated by scientific investigation and employed under medical oversight to avert any possible adverse interactions with traditional therapy.

Recent investigations of alternative therapies have highlighted the potential of *Beta vulgaris* L., a beetroot. *Beta vulgaris* L. belongs to the Chenopodiaceae family and has long been a fundamental component of the traditional western diet [4]. The therapeutic effects of this plant are attributed to its wide range of bioactive components, such as flavonoids, alkaloids, terpenoids, steroids, and betalains [5]. Betalains are pigments exclusive to this group, showing strong antioxidant properties, as evidenced by multiple studies [6]. Several investigations have demonstrated that *Beta vulgaris* has a hematopoietic effect that correlates with its anti-thrombocytopenic effects [7-9]. However, the specific mechanism and association between this activity and the molecule under investigation remain unknown.

The aim of this study was to investigate the mechanistic components through which the phytochemicals present in Beta Vulgaris L. might induce anti-thrombocytopenic effects.

This examination will involve in vitro and in silico investigations that provide insights into molecular-level interactions. Additionally, the antioxidant capacity of beetroot, which is a crucial factor in mitigating platelet dysfunction caused by oxidative stress, was evaluated. Considering the wide range of manifestations and degrees of severity associated with thrombocytopenia, which is particularly prevalent in intensive care units, it is important to investigate natural alternatives that are less hazardous, such as *Beta Vulgaris* L.

This study also involved a computer-based investigation of *Beta Vulgaris L.*, focusing on its phytochemical makeup to discover its potential as a treatment for thrombocytopenia. We explored the process of determining its antioxidant capacity and speculated that its abundant antioxidant profile may play a crucial role in its effectiveness as a treatment for thrombocytopenia. Ultimately, this research seeks to connect traditional knowledge with contemporary scientific approaches to gain a thorough understanding of the medicinal capabilities of *Beta Vulgaris* L. The combination of traditional knowledge and modern methods is expected to lead to innovative therapies in the fight against thrombocytopenia and related problems.

MATERIALS AND METHODS

The materials used were 96% ethanol (Merck), gallic acid (Sigma) ,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma), Standard Betanin (Sigma) $AlCl_3$ (Merck), Folin–Ciocalteu (Sigma), Quercetin (Sigma), distilled water, sodium acetate (Merck), and sodium bicarbonate (Merck), citric acid (Merck) and Ascorbic acid (Merck). The tools used are laboratory glassware, microtube, rotary evaporator (Mingyi), UV-Vis Spectrophotometer (Peak).The animals used were rats weighing 150-180 grams obtained from the Faculty of Pharmacy, Universitas Sumatera Utara.

Sample Collection

Beetroots were obtained from a local market in Padang Bulan, North Sumatra, Indonesia with an authentication number by Herbarium Medanense: 042/MEDA/2022.

Extraction Method

The fresh beetroot was sliced into small pieces, placed in a drying cabinet (40ºC), dried samples then crushed, and sieved through an 80-mesh sieve to obtain dried powder. Furthermore, 50 g of dry

powder was placed in the reflux apparatus, followed by the addition of 300 ml of 96% ethanol. Additionally, 0.5% citric acid and 0.1% ascorbic acid of the total solvent used were used. Following the soaking process, the combination of the sample and solvent was subjected to reflux at 78 °C for a duration of two hours. The residual substance obtained from the original extraction was subjected to a second extraction using 150 mL of the solvent, employing the same technique and solvent. Continuous extraction was carried out by repeatedly utilizing the same residue and 150 mL of solvent until the color of the resulting filtrate reached a steady state. The liquid that passed through the filter was removed using a rotary evaporator (Mingyi Model: RE-52A) until a concentrated substance was formed, the percent yield obtained were 63, 3% [10].

Identification of Phytochemical Contents

The ethanol extract of beetroot was subjected to phytochemical screening to analyze the presence of secondary chemical metabolites such as alkaloids, flavonoids, glycosides, tannins, saponins, terpenoids, and steroids [11, 12].

Calculation of Total Phenol and Flavonoid Concentration

Folin reagent was used to measure the total phenol concentration (TPC) of the samples. A volume of 100 μL of beetroot extract at a concentration of 500 μg/ml was mixed with 7.9 mL of distilled water and 0.5 mL and Folin-Ciocaleu reagent (diluted at a ratio of 1:10 v/v) for 1 min using a vortex mixer. Following the mixing process, a solution consisting of 1.5 mL of sodium bicarbonate dissolved in water at a concentration of 20% was introduced into the mixture. The mixture was then left undisturbed for 90 min with periodic shaking. The absorbance at 775 nm was quantified using a spectrophotometer. The total phenolic content was calculated by determining the amount of gallic acid equivalent in milligrams per gram of the extract. A methanol solution was used as a blank. The experiments were conducted in times [13]. Spectrophotometry and colorimetric techniques were used to determine the total flavonoid concentration. The ethanol extract derived from beetroot was dissolved in 25 mL methanol and further diluted to a concentration of 300 parts per million (ppm). A 2 mL aliquot of the sample, which had a concentration of 300 parts per million (ppm), was combined with 0.1 mL $AlCl_3$, 0.1 mL of sodium acetate, and 2.8 mL of purified water. The absorbance at 750 nm was quantified using a visible spectrophotometer. The flavonoid concentration was quantified by measuring the amount of quercetin per gram of material (mg Q/g) [14].

Determination of DPPH Radical Scavenging Activity

In summary, 1 mL of the test sample at each concentration (100, 50, 25, 22.5, and 6.5 μg/mL) was combined with 1 mL of the DPPH solution with concentration 200 μg/ml. Subsequently, 3 mL methanol was added. The mixture was mixed thoroughly until evenly distributed and then placed in a dark room for 30 min. Absorbance was measured using a UV-Vis spectrophotometer (PEAK E-1000UV) at a wavelength of 513 nm. Furthermore, quercetin (100 μg/mL) were use as comparative standard. The antioxidant activity value (IC_{50}) was determined by calculating a linear regression equation that relates the percentage of inhibition to the concentration or fraction of the sample. The concentration is represented on the x-axis, whereas the percentage of inhibition is represented on the y-axis. To construct the regression equation $y = bx + a$. Subsequently, the y value was substituted with a numerical value of 50. IC 50 is the concentration of the sample required to block 50% of the DPPH radical [15].

Determination of Betanin content by Thin Layer Chromatography

Betanin compounds were identified in the beetroot extract using thin layer chromatography on a silica plate G60 F254 (10 cm × 10 cm) with a mobile phase consisting of acetonitrile: methanol: water: glacial acetic acid (2:7:1:0.1). The plate was produced in a vessel by chromatography until it reached the limit on the TLC plate, with a top border of 0.5 cm and a bottom limit of 1.5 cm. The dried plate was then used to spot a third type of extract, consisting of ethanol, beetroot, and betanin standards, onto TLC plates. The plate was placed in a vessel for chromatography that contained a saturated mixed solvent, sealed, and eluted until the plate reached its limit. Subsequently, the plate was elevated and air-dried. The plates were examined under UV light at wavelengths of 254 and 366 nm to observe the formation of chromatogram stains [16].

***In-Vitro* Hemostatic Activity Test**

The clotting time and microscopic appearance of clotted blood in rat whole blood were assessed using the Lee-White and Eustrek methods [17]. Male rats weighing 150-200 grams were categorized into five groups and rendered unconscious using diethyl ether. A 2.5 ml blood sample was obtained from the retroorbital sinus. Each group consisted of 5 tubes. The normal group consisted of 0.5 ml of blood, whereas the negative control group consisted of 0.5 ml of EDTA 15% solution, to which 0.5 ml of blood was added. The treatment group comprised the inclusion of beetroot extracts at concentrations of 0.5%, 1%, and 2%. Upon introducing blood into each tube, a stopwatch was used to ascertain the duration of clot formation. Every 30 s, each tube was tilted, and the formation of a blood clot was observed for a duration of 2 h until coagulation or clotting occurred. The experimental protocol was authorized by the Animal Research Ethics Committees of Universitas Sumatera Utara under approval number 0316/KEPH-FMIPA/2022.

Microscopic Examination by Eustrek method

Five pieces of the optical glass were prepared. Following the completion of the Lee White technique, a single droplet of blood was extracted from each group and placed onto an object glass. The edge of the lens of a different object is utilized to disperse the blood droplet.

The blood clot was observed using a microscope at a magnification of 40 [18].
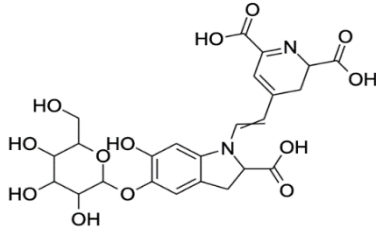
In-silico Tools

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, Protein Data Bank for accessing protein structure data, PubChem for accessing information on chemical compounds, and SwissDock for conducting protein-ligand docking simulations.

Preparation of ligands and proteins

The thrombopoietin receptor (TpoR) gene 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 the 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 mol2 format. Molecular docking involves the interactions between proteins and either test chemicals or natural ligands. SwissDock platform was used to execute the docking procedure. Quantification of docking data was conducted using the Gibbs free energy (ΔG) value [19]. Table 1 lists the precise attributes of these ligands.

Table 1: Ligand name

Name	Formula	Chemical Structure
Betanin	$C_{24}H_{26}N_2O_{13}$	

Rendering of docking outcomes

The visualization process was carried out using the USCF Chimera 1.16.The 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 saved in *. png file format [20].

RESULTS AND DISCUSSION
Qualitative Phytochemical Identification Result

The phytochemical screening results showed that the reflux extract of beetroot contained flavonoids, alkaloids, saponins, tannins, glycosides, and steroids/terpenoids.

Additionally, thin-layer chromatography (TLC) was used to determine the presence of betanin in the beetroot samples, comparing it to a standard betanin. Standard samples and betanin were dissolved in ethanol. They were then applied to a thin-layer chromatography

(TLC) plate using a capillary tube. Subsequently, they were introduced into a chamber containing saturated and eluted phase motions.

Table 2: Phytochemical Identification Results

Content	Reflux Extract
Flavonoids	+
tannins	+
Saponins	+
Steroids / terpenoids	+
Glycosides	+
Alkaloids	+

The shooting distance for comparing the sample and standard was 2 cm, whereas the lower and upper limits for elution were 1.5 cm and 0.5 cm, respectively. Another stain was identified by UV light at a wavelength of 254 nm. The thin-layer chromatography pattern obtained from reflux and MAE of beetroot extract, as well as the standard betanin, exhibited the same pattern. The distance between the stain and eluent in both cases was 4 and 8 cm, respectively. The resulting RF value was 0.5, as shown in figure 1 and table 3.

Figure 1: Betanin Chromatogram (R: Reflux extract; S: Standard of Betanin)

Table 3: Thin layer chromatography results

Sample	Amount stain	Spot distance(cm)	Track length phase motion (cm)	R_f value
Reflux Extract	1	4	8	0.5
Betanin Standard	1	4	8	0.5

Determination of Total Phenolic, Flavonoid Contents and Antioxidant Activity

The majority of the chemical components found in plants are phenolic compounds. The total phenolic content of the extracts was determined by spectrophotometry using the Folin-Ciocalteu reagent. Gallic acid was used as a reference phenolic component for comparison. The equation of the line derived from the results was $Y = 0.01486x + 0.03466667$, with $r2 = 0.9919$. The ethanol extract of beetroot had a total phenolic content of 312.2 mg GAE/g extract. The calibration curve for quercetin was determined using the equation

$y = 0.01391429x + 0.04180953$ with $r2 = 0.993$. The total phenolic and flavonoid content is shown in tables 4 and 5.

Table 4: Results of Total Phenol Content

Sample	Rate Phenol Total (mg GAE/g sample)	Mean (mgGAE /g sample)
Extract Reflux Method	39.48	39.72 ± 2.56
	42.40	
	37.29	

Table 5: Results of Total Flavonoid Content

Sample	Flavonoid Levels Total (mg QE/g sample)	Mean (mg QE/g sample)
Extract Reflux Method	5.23	5.47 ± 0.39
	5.93	
	5.26	

The IC50 values were used to interpret the results of the DPPH assay. IC50 refers to the concentration of the substrate that results in a 50% reduction in DPPH activity [21]. The plant samples were assessed for their ability to donate hydrogen using DPPH, a stable free radical centered on nitrogen that causes a deep purple color in a methanol solution. This measurement determined the antiradical power of the material. Antioxidants have the ability to either donate an electron or a hydrogen atom to DPPH, hence counteracting its free radical nature [22]. A low half maximum inhibitory concentration (IC50) value indicates a high level of antioxidant activity in the fraction, as it requires a low dose to inhibit 50% of radical oxidation. The results of the IC50 investigation in the Beta vulgaris extract are displayed in Table 6, indicating a strong IC50 value of 95.71 μg/mL.

A low half maximum inhibitory concentration (IC50) value indicates a high level of antioxidant activity in the fraction, as it requires a low dose to inhibit 50% of radical oxidation. The results of the IC50 investigation in the Beta vulgaris extract are displayed in Table 6, indicating an IC50 value of 95.71 μg/mL.

Table 6: Antioxidant activity of Reflux extract of Beetroot

Sample	IC50
Reflux Extract	95.71 μg/ml
Control (Quercetin)	1.097 μg/ml

Antioxidants exhibited high potency when the IC50 value was below 50 μg/ml, moderate potency when the IC50 value was between 50-100 μg/ml, and low potency when the IC50 value exceeded 150 μg/ml. There is an inverse relationship between antioxidant activity and the IC50 value, meaning that as antioxidant activity increases, the IC50 value decreases [23].

Microscopic coagulation

The Lee-White method was employed to microscopically examine the state of blood cells after a 2-hour observation period. A single droplet of blood was extracted using a pipette and placed on the glass object. Subsequently, a blood smear was created by gently touching the blood droplet on the object glass with another glass object, causing the droplet to spread. The spreading motion is then continued in the opposite direction towards the other side of the object glass, pulling it until it forms a tongue-like shape and a thin layer. Figure 2 and Table 7 display the results of microscopic observation of blood cell morphology using a suspension of beetroot extract.

Figure 2: Blood microscopic observation, A= Normal Group; B= Negative Group; C= Blood + EDTA + Beetroot extract 0.5 %; D: Blood + EDTA +Beetroot extract 1%; E= Blood + EDTA + Beetroot extract 2%.
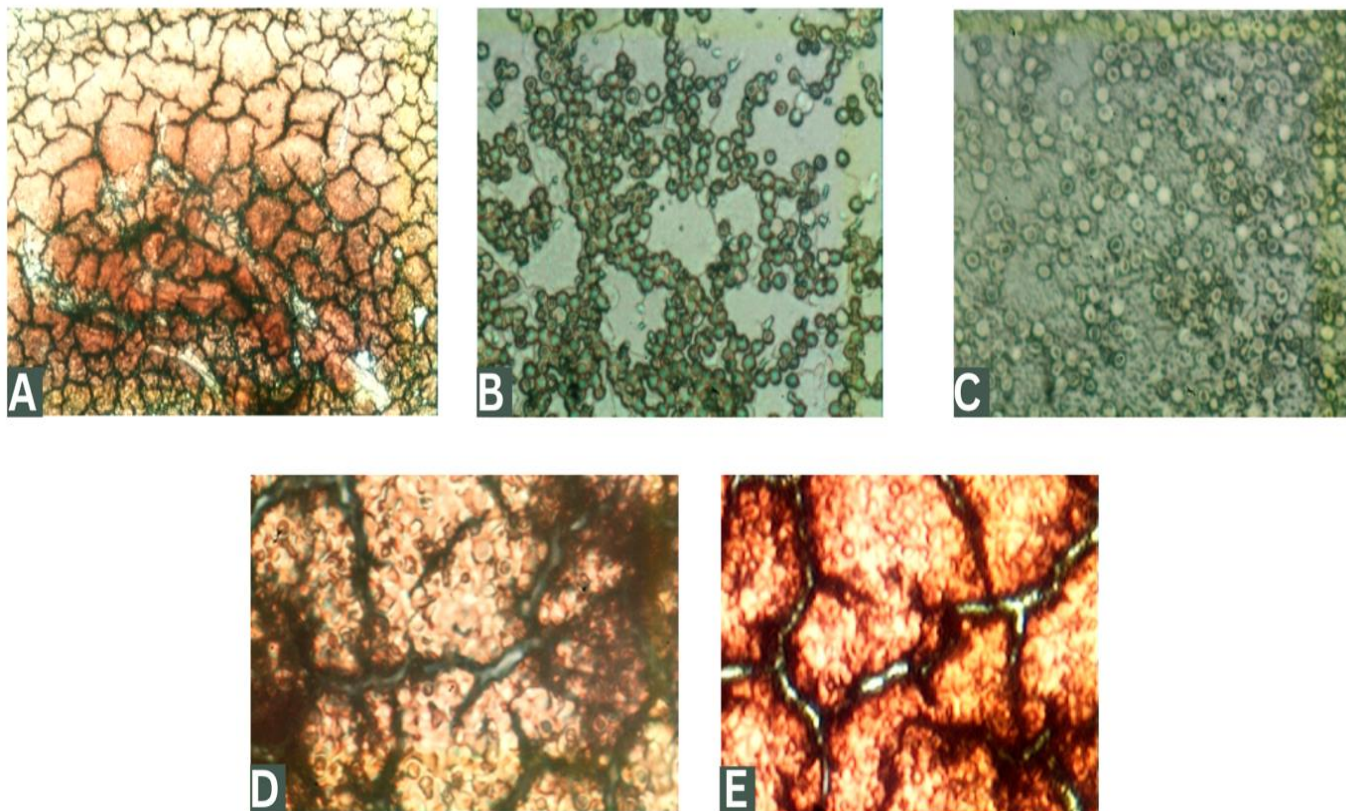
Table 7: Results of microscopic observation of blood cell shape with 5 different treatments

Group	Observation result
Normal (0.5 ml blood)	Clumped blood cells are characterized by blood cells sticking together to form a dense group with each other and experiencing lysis (the red blood cells break apart and become shapeless). round)
Negative Control (Blood + EDTA)	Blood cells do not clot, characterized by blood cells that are not attached to each other and have a normal shape
Blood + EDTA + Beetroot extract 0.5 %	Blood cells do not clot, characterized by blood cells that are not attached to each other and have a normal shape
Blood + EDTA + Beetroot extract 1%	blood cells are characterized by visible blood cells stick together and some blood cells lyse
Blood + EDTA + Beetroot extract 2%	blood cells are characterized by visible blood cells stick together and some blood cells lyse

The alterations in the appearance of blood cells in figure 3 following the addition of beetroot extract indicate that the compounds present in the extract might have the ability to promote blood clotting or provide protection to red blood cells. These effects could be attributed to the antioxidant characteristics of beetroots. This work utilizes the Lee-White method to examine microscopic samples, effectively connecting a conventional coagulation test with contemporary investigative hematology techniques [24, 25]. In addition, the study's novel methodology prompts inquiries into the interplay between natural chemicals and anticoagulant drugs such as EDTA, which are frequently employed in laboratory environments. This interaction is particularly intriguing as it could provide valuable insights into the possible use of natural chemicals in altering coagulation pathways or safeguarding blood cells during stressful settings [25]. Although this study has promising implications, it is not exempt from constraints. When evaluating the results, it is important to consider the brief observation duration and possible impact of EDTA on cell shape. Hence, future investigations should strive to duplicate these discoveries over prolonged durations and devoid of anticoagulants that could impede the coagulation process.
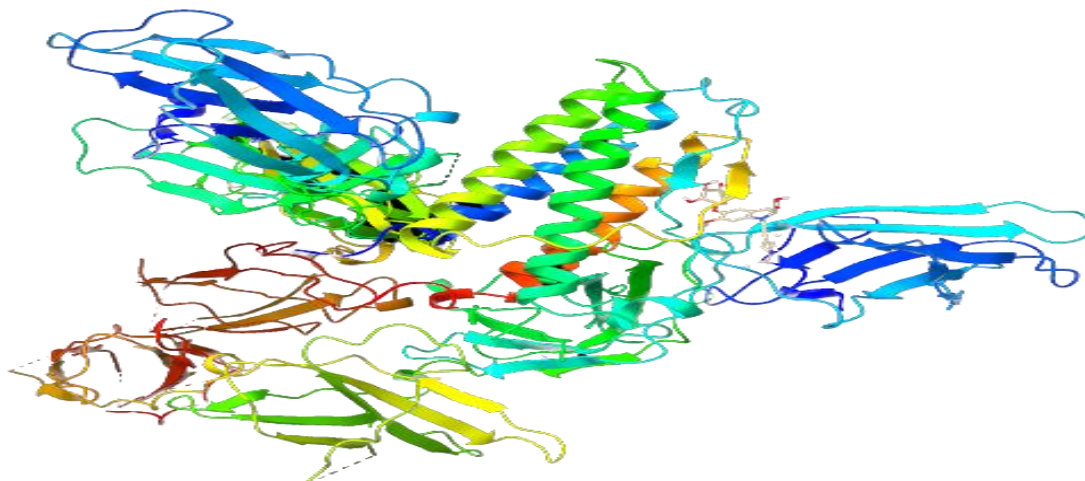
TpoR Docking analysis

The Thrombopoietin Receptor (TpoR), sometimes referred to as c-Mpl, is a protein that is mostly found on the surface of platelet precursors located in the bone marrow. It plays a crucial role in the regulation of thrombopoiesis and in the mechanism responsible for platelet production. Thrombopoietin (TPO) is the hormone that stimulates the formation of platelets by binding to TpoR. When TPO binds to TpoR, it triggers a series of intracellular signaling pathways that stimulate the growth and multiplication of megakaryocytes, which are the cells responsible for releasing platelets into the bloodstream. Abnormalities in TpoR activity can disturb platelet generation. Reduced Tpo or TpoR activity can result in thrombocytopenia, a disorder defined by a diminished number of platelets, which in turn increases the risk of bleeding [26]. On the other hand, excessive activation of TpoR can lead to thrombocytosis, a condition characterized by excessive production of platelets, which might potentially increase the likelihood of thrombosis [27].

Computational docking can be employed to examine the interaction between various chemicals and TpoR, particularly in studies focused on the potential of betanin, a molecule derived from beets, as a TpoR agonist. This technique enables scientists to model and depict the strength of the interaction between betanin and TpoR, aiding in the prediction of the capacity of betanin to activate thrombopoiesis. The outcomes of these docking investigations are displayed in tables and figures to demonstrate the effectiveness of binding and the potential arrangement of the betanin-TpoR interaction. The docking results are presented in Table 8 and Figure 3.

Table 8: Docking affinity scores - kcal/mol

Ligand	Protein	ΔG (kkal/ mol)	Amino Acid Residue
Betanin	Thrombopoietin Receptor (TpoR)	-8.1	LEU62, PRO63, ALA64, VAL65, ASP66, ARG138, THR139 THR140, ALA141, HIS142, ALA147, ILE148, LEU150, SER151, HIS154, PRO70, ARG71, ARG102, PHE104, PHE105, PRO106, PHE126.

Figure 3: Betanin-TpoR Docking Visualization

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The primary objective of our study was to investigate the binding interactions between betanin and thrombopoietin receptor (TpoR), with a specific focus on betanin. Molecular docking analysis yielded a binding affinity of -8.1 kcal/mol for betanin, indicating a possible stable association with TpoR. This information is summarized in Table 6. A comprehensive study can be conducted based on research findings and accessible information regarding thrombopoietin (TPO), which is a natural ligand for the thrombopoietin receptor (TpoR). In this study, we primarily examined the binding interactions between Betanin and TpoR. It reveals a binding affinity of -8.1 kcal/mol, suggesting a possibly stable relationship that is similar to the interaction between TPO and TpoR. TPO plays a vital role in megakaryocyte development and platelet production by interacting with CD110, a protein encoded by the c-mpl gene.The interaction of CD110 results in the formation of dimers and subsequent phosphorylation of many kinases and receptors, highlighting its significance in the process of platelet formation.

Betanin binds to an interaction network comprising of the following amino acids: LEU62, PRO63, ALA64, VAL65, ASP66, ARG138, THR139, THR140, ALA141, HIS142, ALA147, ILE148, LEU150, SER151, HIS154, PRO70, ARG71, ARG102, PHE104, PHE105, PRO106, and PHE126. These specific interaction points may be crucial for the activation or inhibition of the betanin receptors. By juxtaposing these data with the interaction sites of TPO and TpoR, one could gain valuable insights into the ways in which betanin's impact on TpoR-mediated pathways may resemble or diverge from that of Tpo.

Comparison of the binding sites of betanin and thrombopoietin (Tpo) on the thrombopoietin receptor (TpoR) revealed that each compound interacted with distinct amino acid residues. A Betanin study revealed an extensive interaction network involving residues such as LEU62, PRO63, ALA64, VAL65, and ASP66. In contrast, TPO's binding site of TPO on TpoR, specifically within Mpl-EC domain 1 (residues 206-251), involves critical residues, such as Leu(228), Leu(230), Asp(235), and Leu(239)[28].This difference in binding sites may influence how each ligand modulates TpoR activity, with potential therapeutic implications in regulating megakaryocyte proliferation and platelet formation. This expanded binding-site repertoire suggests that there may be a broader spectrum of interaction patterns that confer phytochemicals with the ability to engage with TpoR with sufficient stability. This could potentially be exploited in the design of novel TpoR-targeting drugs with enhanced specificity and efficacy. Furthermore, an investigation into the interactions of mimic feline thrombopoietin, a protein present in cats that is similar to human thrombopoietin, focused on particular amino acid sites: Thr 213; Ala 211; and Arg 212 [29]. Thrombopoietin, an essential glycoprotein hormone primarily produced in the liver and kidneys, regulates platelet production in the bone marrow. The present study additionally emphasizes the comparable binding sites of betanin and feline thrombopoietin, thereby emphasizing a significant parallel in their biochemical configurations and functionalities.

Despite the structural variations among betanins, it is notable that betanin retains substantial docking affinity, thereby implying the existence of a distinct binding mechanism capable of modulating TpoR. Additional investigations are required to examine the potential therapeutic implications and pharmacodynamic effects of these novel binding interactions on betanin and similar medications, with a specific focus on thrombopoiesis-stimulating pharmaceuticals. Experimental validation of these computational predictions through site-directed mutagenesis and in vitro binding experiments should be the primary objective of subsequent studies. The purpose of these experiments was to ascertain the role of the recently identified residues in signal transmission and TpoR activation. A thorough understanding of these molecular interactions is critical to advance our knowledge of TpoR function and to develop innovative therapeutic agents that specifically target thrombopoiesis.

CONCLUSION

It concluded that the compound betanin, found in *Beta vulgaris* L. extract, demonstrated anti-thrombocytopenia effects in both in vitro and in silico studies.

Conflict of interest

The authors declare that they do not own any conflicts of interest, be it financial, personal, authorship-related, or otherwise, that could potentially influence the research and its findings provided in this publication.

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