DOI: 10.55522/jmpas.V13I1.5882 ISSN NO. 2320 – 7418

International peer reviewed open access journal

Journal of Medical Pharmaceutical and Allied Sciences

Journal homepage: www.jmpas.com CODEN: JMPACO

Research article

Assessing the toxicity and anti-anemia of *Beta Vulgaris L.: in silico*, phytochemical and antioxidant analysis

Marianne Marianne[1], Sony Eka Nugraha*[2], Resta Yolanda Amelia[3] , Petra Sri Etika Laia[3]

[1]Department of Pharmacology and Clinical/Community Pharmacy, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia
[2]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia
[3]Undergraduate Program, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia

Corresponding author: Sony Eka Nugraha, ✉ sonyekanugraha@usu.ac.id, **Orcid Id**: https://orcid.org/0000-0001-8849-2481

Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia

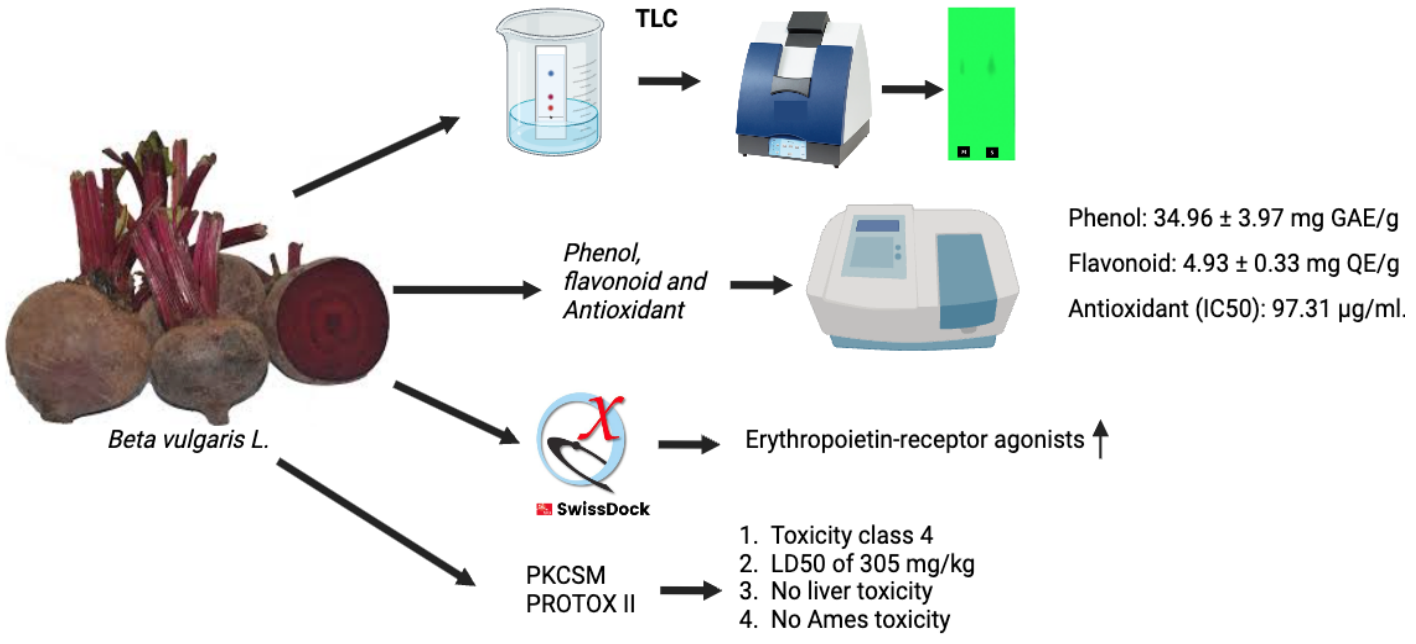
Received - 08-11-2023**, Revised** - 17-01-2024, **Accepted** - 28-01-2024 **(DD-MM-YYYY)**

Refer This Article

Marianne Marianne, Sony Eka Nugraha, Resta Yolanda Amelia, Petra Sri Etika Laia, 2024. Assessing the toxicity and anti-anemia of *Beta Vulgaris* L.: in silico, phytochemical and antioxidant analysis. Journal of medical pharmaceutical and allied sciences, V 13 - I 1, Pages - 6285 – 6291. Doi: https://doi.org/10.55522/jmpas.V13I1.5882.

ABSTRACT

The objective of this investigation was to determine the toxicity, anti-anemia activity, betanin content, flavonoid content, and antioxidant capacity of *Beta vulgaris* L. Microwave-assisted extraction (MAE) was implemented to optimize the extraction procedure. The total phenolic content (TPC) and total flavonoid content (TFC) of the extracts were determined using the Folin-Ciocalteu colorimetric and aluminum chloride methods, respectively. Furthermore, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was employed to ascertain antioxidant capacity, whereas thin-layer chromatography was used to identify betanin content. The toxicity and anti-anemia properties were assessed in silico. The in-silico methods encompass several steps: ligand and target protein synthesis, docking of betanin compounds with the erythropoietin receptor (EpoR) target protein via SwissDock, docking data visualization via USCF Chimera, and toxicity analysis via the pkCSM and Protox online tools.



The findings indicated that the beetroot extract contained a total of 34.96 ± 3.97 mg GAE/g sample of phenol. The flavonoid content of beetroot was 4.93 ± 0.33 mg QE/g sample. Qualitative analysis conducted using thin-layer chromatography yielded results indicating the presence of betanin in the extract. The IC50 value for the antioxidant activity was 97.31 µg/ml. The docking study revealed the effective interaction of betanin with several amino acid residues, suggesting a low toxicity potential, supported by toxicity class 4 and an LD50 of 305 mg/kg, and the absence of liver and Ames toxicity. It concluded that betanin is a promising anti-anemia agent. levels.

Keywords: Antioxidant, Anemia, Betanin, Insilico, Toxicity.

INTRODUCTION

Anemia is a medical condition marked by adeficiency of red blood cells and a lower than normal level of hemoglobin in the bloodstream. It is a significant public health issue that affects both developed and developing nations worldwide [1]. The most prevalent forms of iron deficiency anemia are chronic inflammation, severe hemorrhage, and inadequate dietary intake, all of which contribute to persistently low iron levels [2]. Among the numerous interventions and treatments for anemia, there is a growing interest in exploring natural therapies, specifically those involving plant-derived compounds. The increasing interest in natural medicines stems from their potential to provide effective treatment with minimal negative side effects, thereby improving patient compliance and overall therapeutic outcomes [3].

Beta vulgaris L., commonly known as beetroot, has been consumed not only for nourishment but also because of its conceivable therapeutic attributes.Throughout history, beetroots have been used as a remedy for a variety of ailments, including constipation, fever, and dermatological problems. Recent scientific research has begun to unveil its potential therapeutic benefits, such as its ability to improve cardiovascular health, inflammation, and hematopoiesis [4-6].The antioxidant activity of *Beta vulgaris* L. further underscores its therapeutic effect. Anemia has been found to be correlated with increased levels of oxidative stress; therefore, the antioxidant properties of potential treatment medications are of utmost importance [7]. *Beta vulgaris* L has been shown to possess anti-anemia properties in several studies [8-10]. However, the mechanism and correlation of this activity with the compound in question remain unknown.

The term "In Silico" refers to the execution of tasks through the use of computer-based methodologies [11].In biomedical research and medication development, in silico methods are expecting the efficacy, mechanism, and behavior of possible therapeutic drugs before they are tried in biological systems. By avoiding as many physical experiments as feasible, this reduces expenses and speeds up research [12]. The aim of this computer-based study was to assess the therapeutic potential of *Beta Vulgaris* L. in treating anemia. This can be achieved by studying its phytochemical composition and elucidating the precise mechanisms involved in its anti-anemia activity [13]. The current research investigated the procedure for ascertaining its antioxidant capacity and hypothesized that its rich antioxidant profile might significantly contribute to its efficacy as a treatment for anemia. The overarching objective of this research is to establish a bond between traditional wisdom and modern scientific methodologies in order to acquire a comprehensive understanding of the therapeutic properties of *Beta vulgaris* L.The integration of conventional wisdom and contemporary approaches is anticipated to Yield groundbreaking therapeutic interventions for anemia and its associated complications.

MATERIALS AND METHODS

Sample Collection and Extraction Method

Beetroots were collected from a local market in Padang Bulan in North Sumatra, Indonesia with an authentication number by Herbarium Medanense: 042/MEDA/2022. Modified microwave (SAMSUNG, ME731KCeramic Inside, and Indonesia) was utilized to extract beetroot. Microwave-assisted extraction was started by weighing 15 g of dried beetroot powder, then dissolving in 96% ethanol at a 5:1 ratio (w/w) of solvent to sample, with the addition of 0.5% citric acid and 0.1% ascorbic acid. The mixture was stirred and allowed to rest for 20 min. Microwaves were set up with a 15-minute duration and 180 W of power, and the extraction procedure was carried out. Whatman filter paper no. 1 was used to obtain the filtrate from the extraction findings. The filtrate was collected and evaporated using a rotary evaporator until a thick extract [14].

Identification of Phytochemical Contents

The phytochemical content of alkaloids, flavonoids, glycosides, tannins, saponins, terpenoid, and steroids were examined as part of the phytochemical screening of the ethanol extract of beetroot [15, 16].

Calculation of Total Phenol and Flavonoid Concentration

Folin's reagent was used to determine the total phenol concentration in the sample (TPC). Beetroot extract (100 µl, 500 µg/ml) was combined with 7.9 mL of distilled water and 0.5 mL of Folin-Ciocaleu reagent (1:10 v/v) for 1 min using a vortex mixer. After mixing, 1.5 mL of 20% aqueous sodium bicarbonate was added to the mixture, which was allowed to sit for 90 min with intermittent shaking. The absorbance at 775 nm was measured using a spectrophotometer (Peak Intrument™Model E1000). The total phenolic content was computed using milligrams of gallic acid equivalent per gram of the extract. A methanol solution was used as a blank. All experiments were performed in triplicate [17]. The total flavonoid concentration was determined using a spectrophotometer and colorimetric method. The beetroot ethanol extract was dissolved in 25 mL methanol and subsequently diluted to a concentration of 300 ppm. A sample (2 mL) with a concentration of 300 ppm was mixed with 0.1 mL of AlCl3, 0.1

milliliters of sodium acetate, and 2.8 mL of purified water. Absorbance was measured at 750 nm using a visible spectrophotometer. The flavonoid content was determined using equal milligrams of quercetin per gram of material (mg Q/g) [18].

Determination of DPPH Radical Scavenging Activity

Briefly, 1 mL of the test sample from each concentration (100, 50, 25, 22.5, and 6.5 µg /mL) was added to 1 mL of DPPH solution. Subsequently, methanol (3 mL) was added to the mixture. The mixture then incubated in a dark room for 30 min. Absorbance was measured using a UV-Vis spectrophotometer at 513 nm. The value of antioxidant activity (IC_{50}) was calculated based on the linear regression equation between % inhibition and the sample concentration or fraction, where the x-axis is the concentration and the y-axis is the % inhibition. Thus, the regression equation y = ax + bis obtained [19].

Determination of Betanin Content by Thin Layer Chromatography

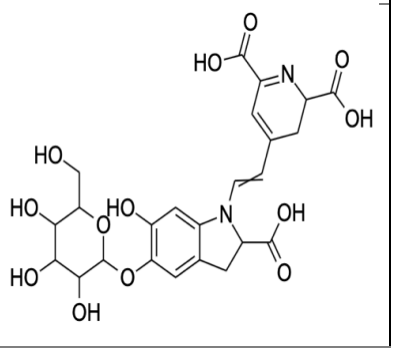
Betanin components in beetroot extract were identified using thin-layer chromatography on a 10 cm × 10 cm silica plate and a 2:7:1:0.1 phase motion acetonitrile: methanol: water: acid acetate glacial solution. The plate was developed in vessel chromatography till the TLC plate limit (border-top: 0.5 cm; limit bottom: 1.5 cm). TLC plates were spotted with ethanol beetroot extract and betanin standards. The plate was placed in a saturated solvent combination in an in-vessel chromatograph and eluted until the limit was achieved. The plates were raised and air-dried and plate stains were observed [20].

In Silico Tools

A 14-inch HP laptop with 14GB RAM, 256GB SSD, Windows 11, and 64-bit chipset was used. This study used Chimera 1.16 to visualize molecules, the Protein Data Bank for chemical compound information, Swiss ADME for drug-like properties, Swiss Dock for protein-ligand docking, the web server pkCSM for binding affinity, and the Protox online tool for toxicity.

Preparation of Ligands and Proteins

Table 1: Ligand name

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

The PDB selection criteria for choosing a suitable resolution are denoted by 1ERN. This identifier refers to the detailed structure of the extracellular domain of the EPO receptor, as per the Protein Data Bank (www.rcsb.org). This information aids in understanding its classification in the cytokine group. Subsequently, the UCSF Chimera 1.16 tool was used to prepare the sample by eliminating residues. The docking results were quantified using Gibbs free energy (ΔG) value [21]. Furthermore, details of the ligands are shown in table 1.

Rendering of Docking Outcomes

The visualization process was carried out using the USCF Chimera 1.16. This was performed by inputting the target protein data in the form of a *. PDB file, as well as the docking results for the same *. PDB file format. The visualization results are stored in *. Png file format [21, 22].

Toxicity Prediction

The pkCSM web server was used to investigate Ames toxicity, hepatotoxicity, and skin sensitization.The Protox online tool was used to predict the LD50 and toxicity classes [23].

RESULTS AND DISCUSSION

Qualitative Phytochemical Identification Result

The phytochemical screening results showed that the MAE extract of beetroot contained flavonoids, alkaloids, saponins, tannins, glycosides, and steroids/terpenoids (Table 2).

Table 2: Phytochemical Identification Results

Content	MAE Extract
Flavonoids	+
Tannins	+
Saponins	+
Steroids/terpenoids	+
Glycosides	+
Alkaloids	+

Determination of Total Phenolic and Flavonoid Contents

The equation of the line produced based on the results was Y= 0.01486x + 0.03466667 (r2 = 0.9919). The ethanol extract of beetroot had a total phenolic content of 312.2 mg GAE/g extract. The quercetin calibration curve was calculated using the equation y = 0.01391429x + 0.04180953; and (r2 = 0.993). The total phenolic, flavonoid content and antioxidant are shown in Table 3.

Table 3: Results of total phenol content, flavonoid, and antioxidant activity

Phenol Total (mgGAE/g sample)	Flavonoid Levels Total(mg QE/g sample)	DPPH Antioxidant Assay (IC50)	
		Beetroot Extract	Control (Quercetin)
34.96 ± 3.97	4.93± 0.33	97.31 µg/ml	1.097 µg/ml

The total phenol content of beetroot obtained from the ethanol extract was 34.96±3.97 mg GAE/g sample, respectively. Furthermore total flavonoids 4.93±0.33 QE/g sample. Phenolic compounds are distinguished by the presence of one or more hydroxyl groups connected to at least one aromatic ring that plays role as an antioxidant [24].Additionally, the IC_{50} value is a metric that is used to interpret the results of the DPPH technique [25]. Table 3 shows the results of the IC_{50} investigation in *Beta vulgaris* extract was 37.66 µg /mL. Antioxidants were considered very strong if the IC50 value was less than 50 µg/ml, strong if the IC50 was 50-100 µg/ml, moderate if the IC50 was 100-150 µg/ml, and weak if the IC50 was greater than 150 µg/ml. The higher the antioxidant activity, the lower the IC50 value [23].

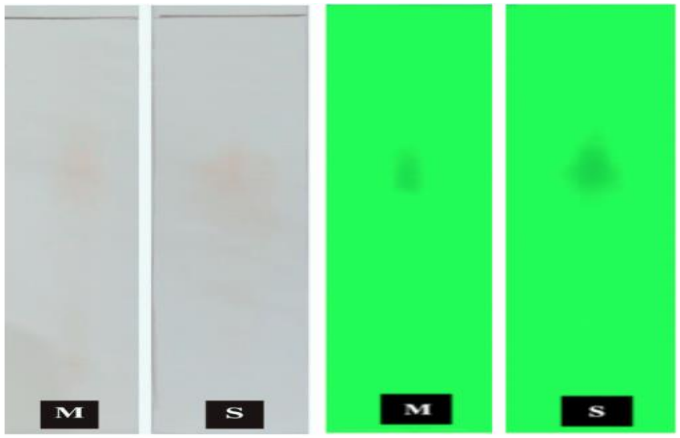
Betanin Content Analysis

Table 4: Thin layer chromatography results

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

The thin layer chromatography pattern of beetroot extract, as well as standard betanin, produced the same pattern with distance vines stain = 4 cm and distance vines eluent = 8 cm, resulting in an R_F value = 0.5, as shown in figure 1 and table 4.

Figure 1: Betanin Chromatogram (M: MAE extract; S: Standard of Betanin)



The stability of betanin pigment is influenced by several factors, such as temperature and pH. This was proven by research conducted by Asra et al. in 2020, where at a temperature of 100°C, the color and drop rate of betacyanin in the beetroot extract occurs. Betanin is a derivative of betacyanin, which is the main component of betacyanin in beetroots [26]. A similar study also reported that the longer the extraction time, the higher the phenolic content [27]. Beetroots contain antioxidant chemicals, specifically polyphenols and folic acid. In vitro experiments have revealed that betalain from red beetroots possesses high antiradical and antioxidant characteristics [28].

In this study, 96% ethanol was used as the solvent, along with citric and ascorbic acids.A more acidic environment causes more betalain pigments to exist, and absorption measurements reveal a growing amount of betalains [29, 30]. Several research reported that citric acid compounds can yield a greater total anthocyanin concentration of 27.7 mg/100 g than acetic acid compounds of 26.4 mg/100 g at the same concentration of 0.75 percent. Other research also reported that lyophilized beet using 0.5% citric acid and 0.1% ascorbic acid as solvents showed a higher concentration of betalains, which means that the extraction of betalains properties in beetroot needs acid addition to increase the betalain content [31, 32].

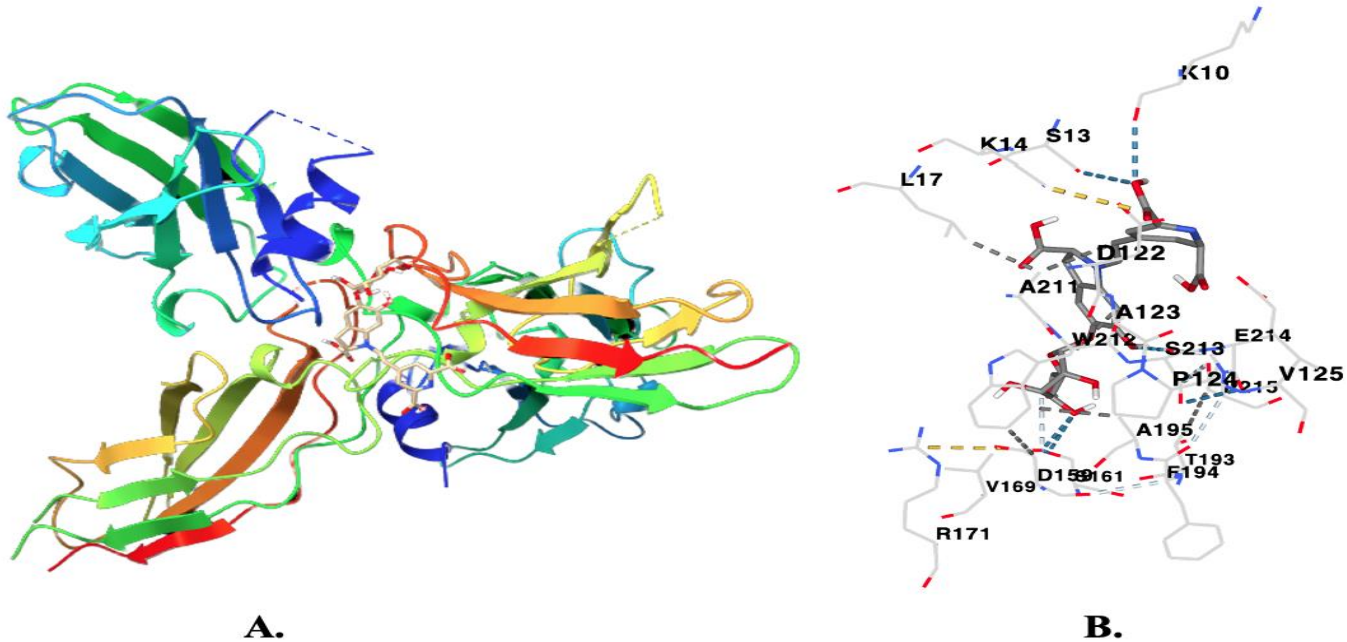
EpoR Docking Analysis

The Erythropoietin Receptor (EpoR) is a protein that resides on the surface of red blood cell precursors in the bone marrow [33]. When erythropoietin binds to its receptor, EpoR, on the surface of erythroid progenitor cells, it triggers a cascade of intracellular signaling events. Disruptions in EpoR function can lead to various blood disorders [34]. The docking results are shown in Table 5 and Figure 2.

Table 5: Docking affinity scores - kcal/mol

Ligand	Protein	ΔG (kkal/ mol)	Amino Acid Residue
Betanin	Erythropoietin Receptor (EpoR)	-7.2	LYS10 SER13 ASP122 ALA123 PRO124 VAL125 GLY126 LEU127 LEU143 PRO146 ALA211 TRP212 SER213 GLU214

Figure 2: Docking Visualization Ligand and Protein, B. Interaction Map Ligand and Protein



Our study focused on the binding interactions between betanin and Erythropoietin Receptor (EpoR). The molecular docking results, as summarized in Table 1, showed a binding affinity of -7.2 kcal/mol for betanin, suggesting a potentially stable interaction with EpoR. Notably, this interaction involves a network of 14 amino acid residues, namely, LYS10, SER13, ASP122, ALA123, PRO124, VAL125, GLY126, LEU127, LEU143, PRO146, ALA211, TRP212, SER213, and GLU214.These binding sites are similar when considering previous reports that identified residues such as LYS10, ARG32, and ARG35 as contributors to EpoR binding [35]. Furthermore, our results did not show direct interactions between Lys 65 and Asn 116, which have been reported to facilitate the interaction of EpoR with Epo [34]. However, a comprehensive understanding of these interactions at the molecular level is essential for furthering our understanding of EpoR function and for the creation of novel therapeutic drugs that specifically target erythropoiesis.

Table 6: Prediction of betanin toxicity (pKCSM)

Property	Model Name	Predicted Value of Betanin	Unit
Toxicity	AMES toxicity	No	Numeric (log ml/min/kg)
Toxicity	Max. tolerated dose (human)	0.599	Categorical (Yes/No)
Toxicity	hERG I inhibitor	No	Categorical (Yes/No)
Toxicity	hERG II inhibitor	No	Numeric (log mg/kg/day)
Toxicity	Oral Rat Acute Toxicity (LD50)	2.477	Categorical (Yes/No)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	3.477	Categorical (Yes/No)
Toxicity	Hepatotoxicity	No	Numeric (mol/kg)
Toxicity	Skin Sensitisation	No	Numeric (log mg/kg_bw/day)
Toxicity	*T.Pyriformis* toxicity	0.285	Categorical (Yes/No)
Toxicity	Minnow toxicity	7.663	Categorical (Yes/No)

***In-silico*Toxicity Prediction**

PKCSM and Protox-II are components of a set of computational techniques designed to predict the pharmacological effectiveness and potential hazards of novel chemical compounds at an early stage of pharmaceutical development.The toxicity predictions of this study are shown in table 6 and 7.

Table 7: Betanin toxicity class (protox online)

Parameters	Betanin
Predicted LD 50	305 mg/kg
Predicted toxicity class	Class 4
Average similarity	45.17%
Prediction accuracy	54.26%

Pro-Tox II has advantages, such as predicting the level of oral toxicity, organ toxicity (hepatotoxicity), toxicological endpointsthereby demonstrating the possible molecular mechanisms underlying the response [37]. The toxicity class was defined according to the Globally Harmonized System (GHS) classification system, which is categorized into six classes. Class I (LD50 5), Class II (5 < LD50 50), Class III (50 < LD50 300), Class IV (300 < LD50 2000), Class V (2000 < LD50 ≤ 5000), and Class VI (LD50 > 5000). The higher the LD50 value, the lower the toxicity [38]. Based on Table 7, the LD50 of the compounds ranged from 305 mg/kg; therefore, they were categorized as class IV. The results of this study showed that the betanin toxicity class is class 4, which is included in the dangerous class. The hepatotoxicity parameters indicated that betanin did not induce hepatotoxicity. Furthermore, in the AMES, hERG I inhibitor, hERG II inhibitor, and Skin Sensitization toxicity were not observed.Consequently, experimental validation through in vitro and in vivo studies is essential to substantiate the efficacy of betanin as an EpoR agonist and unravel its pharmacological profile, toxicity, and therapeutic value.

CONCLUSION

Betanin has potential as an in silico EpoR agonist for the treatment of anemia. Further investigations are required to verify these computational results. Betanin also has an LD50 ranging from 305 mg/kg; therefore, it is categorized as toxicity class IV and does not indicate hepatotoxicity.

Conflict of interests the authors state that they have no financial, personal, authorship, or other conflicts of interest that could affect this research and its outcomes.

ACKNOWLEDGMENT

This research was funded by the "Directorate of Research, Technology, and Community Service," Ministry of Education, Culture, Research and Technology Indonesia, under contract numbers 097/E5/PG.02.00/PT/2022 and 06/UN5.2.3.1/PPM/KP-DRTPM/TI/2022. 0

REFERENCES

1. Chaparro CM, Suchdev PS, 2019. Anemia epidemiology, pathophysiology, and etiology in low and middle income countries. Ann N Y Acad Sci. 1450(1), Pages 15-31. Doi: 10.1111/nyas.14092.

2. Camaschella C, 2019. Iron deficiency. Blood. 133(1), Pages 30-33. Doi: 10.1182/blood-2018-05-815944.

3. IslamMR, Rahman MM, Dhar P, et al, 2023. The Role of Natural and Semi-Synthetic Compounds in Ovarian Cancer: *Molecules.28*, Pages 1-27. Doi: 10.3390/ molecules28052070.

4. Mirmiran P, Houshialsadat Z, Gaeini Z, et al, 2020. Functional properties of beetroot (Beta vulgaris) in management of cardio-metabolic diseases. Nutr Metab. 17(1), pages 1-5. Doi: 10.1186/s12986-019-0421-0.

5. Albasher G, Almeer R, Al-Otibi FO, et al, 2019. Ameliorative effect of Beta vulgaris root extract on chlorpyrifos-induced oxidative stress, inflammation and liver injury in rats. Biomolecules. 9(7), pages 1-16. Doi: 10.3390/biom9070261.

6. Najem ZB, Ghaith AM, 2019. Study on the effect of red beetroot (Beta vulgaris L.) juice intake on some hematological parameters in female rabbits. J Pure Appl Sci. 18, Pages 119-125.

7. Guven S, Meral I, Kurdoglu M, et al, 2022. The nutritional habits and relationship between the antioxidant activity and iron deficiency anemia during pregnancy. East J Med. 27(2), Pages 317-24. Doi: 10.5505/ejm.2022.48091.

8. Abd-El-Fattah ME, Dessouki AA, Abdelnaeim NS, et al, 2021. Protective effect of Beta vulgaris roots supplementation on anemic phenylhydrazine-intoxicated rats. Environ Sci Pollut Res. 28, Pages 65731-65742. Doi: 10.1007/s11356-021-15302-6.

9. Gheith I, El-Mahmoudy A, 2018. Laboratory evidence for the hematopoietic potential of Beta vulgaris leaf and stalk extract in a phenylhydrazine model of anemia. Braz J Med Biol Res. 51(11), Pages 1-8. Doi: 10.1590/1414-431x20187722.

10. Fadlilah SH, Suhartomo DM, Yusan RT, et al, 2023. Beetroot (Beta vulgaris L.) And it's Potential as an Anemia Treatment in Pregnancy. Med and Health Journal. 3(1), Pages 114-27. Doi: 10.20884/1.mhj.2023.3.1.9070.

11. ShahidiF, DissanayakaCS, 2023. Phenolic-protein interactions: insight from in-silico analyses–a review. Food Prod. Process. Nutr. 5(1), Pages 1-21. Doi: 10.1186/s43014-022-00121-0.

12. Kapetanovic I, 2008. Computer-aided drug discovery and development (CADDD): in silico-chemico-biological approach. Chem Biol Interact. 171(2), Pages 165-76. Doi: 10.1016/j.cbi.2006.12.006.

13. VaidyaA, JainS, JainS, et al, 2014. Quantitative structure-activity relationships: a novel approach of drug design and discovery. J PharmPharm Sci. 1(3), Pages 219-232. Doi: 10.1166/jpsp.2014.1024.

14. Cardoso-Ugarte GA, Sosa-Morales ME, Ballard T,et al, 2014. Microwave-assisted extraction of betalains from red beet (Beta vulgaris). LWT- Food Sci Technol. 259(1), Pages276-282. Doi: /10.1016/j.lwt.2014.05.025.

15. Farnsworth NR, 1966. Biological and phytochemical screening of plants. J Pharm Sci, 55(3), Pages225-276. Doi: 10.1002/jps.2600550302.

16. Shaikh JR, Patil MK, 2020. Qualitative tests for preliminary phytochemical screening: An overview. Int J Chem Stud. 8(2), Pages 603-608. Doi: 10.22271/chemi.2020.v8.i2i.8834.

17. Syahputra RA, Lie S, Theo S, Nugraha SE, 2021. Antioxidant, Total Phenol, Total Flavonoid, and LC-MS/MS Analysis of Pometia Pinnata Ethanol Extract. In 2021 IEEE International Conference on Health, Instrumentation & Measurement, and Natural Sciences (InHeNce), Pages 1-5. Doi: 10.1109/inhence52833.2021.9537214.

18. Halim B, Syahputra RA, Adenin I, Lubis HP, Mendrofa F, Lie S, Nugraha SE, 2022. Determination of Phytochemical Constituent, Antioxidant Activity, Total Phenol and Total Flavonoid of Extract Ethanol Phyllanthus emblica Fruit. Pharmacogn J. 14(1), Pages63-67. Doi: 10.5530/pj.2022.14.9.

19. Bobo-García G, Davidov-Pardo G, Arroqui C, Vírseda P, Marín-Arroyo MR, Navarro M, 2015. Intra-laboratory validation of microplate methods for total phenolic content and antioxidant activity on polyphenolic extracts, and comparison with conventional spectrophotometric methods. J Sci Food Agric. 95(1), Pages204-209. Doi:10.1002/jsfa.6706.

20. Kowalska T, Sajewicz M, 2022. Thin-Layer Chromatography (TLC) in the Screening of Botanicals–Its Versatile Potential and Selected Applications. Molecules. 27(19), Pages 1-23. Doi: 10.3390/molecules27196607.

21. Martins da Silva AY, Arouche TD, Siqueira MR, et al, 2023. SARS-CoV-2 external structures interacting with nanospheres using docking and molecular dynamics. J Biomol Struct Dyn. 41, Pages 1-6. Doi: 10.1080/07391102.2023.2252930.

22. Pettersen EF, Goddard TD, Huang CC, et al, 2004. UCSF Chimera-a visualization system for exploratory research and analysis. J Comput Chem. 25(13), Pages 1605-1612. Doi: 10.1002/jcc.20084.

23. Pires DE, Blundell TL, Ascher DB, 2015. PkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. J Med Chem. 58(9), Pages 4066-4072. Doi: 10.1021/acs.jmedchem.5b00104.

24. Abbas O, Compère G, Larondelle Y, Pompeu D, Rogez H, Baeten V, 2017. Phenolic compound explorer: A mid-infrared spectroscopy database. Vib. Spectrosc. 92, Pages111-118. Doi: 10.1016/j.vibspec.2017.05.008.

25. Hidayah SN, Hastuti, AAMB, 2023. Comprehensive estimation of measurement uncertainty in determination of antioxidant activity in natural product by 2, 2′-diphenil-1-picrylhydrazyl (DPPH) radical scavenging assay. Chemical Papers, 77, pages 4579–4587. Doi: 10.1007/s11696-023-02808-1.

26. Asra R, Yetti RD, Ratnasari D, et al, 2020. Studi Fisikokimia Betasianin Dan Aktivitas Antioksidan Dari Umbi Bit Merah (Beta vulgaris L.). J Pharm Sci. 3(1), 14-21. Doi: 10.36490/journal-jps.com.v3i1.35.

27. Jorge AJ, De La Garza TH, Alejandro ZC, et al, 2013. The optimization of phenolic compounds extraction from cactus pear (Opuntia ficus-indica) skin in a reflux system using response surface methodology. Asian Pac J Trop Biomed. 3(6), Pages 436-442. Doi: 10.1016/s2221-1691(13)60093-3.

28. Bangar SP, Sharma N, Sanwal N, et al, 2022. Bioactive potential of beetroot (Beta vulgaris). Food Res Int. 158, Pages 111556. Doi: 10.1016/j.foodres.2022.111556.

29. Dai J, Mumper RJ, 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 15(10), Pages7313-7352. Doi: 10.3390/molecules15107313.

30. Khan MI, 2016. Stabilization of betalains: A review. Food Chem. 197, Pages1280-1285. Doi: 10.1016/j.foodchem.2015.11.043.

31. Widyasari MAA, 2002. Apple polyphenolics and their antioxidant properties: influence of cultivars, post-harvest storage, and 1-MCP treatment.

32. Neagu C, Barbu V 2014. Principal component analysis of the factors involved in the extraction of beetroot betalains. J Agroalimentary Process Technol, 20(4), Pages 311-318. Doi: 10.1556/aalim.2015.0002.

33. Watowich SS, 2011. The erythropoietin receptor: molecular structure and hematopoietic signaling pathways. J Investig Med. 59(7), Pages 1067-72. Doi: /10.2310/jim.0b013e31820fb28c.

34. Tang P, Wang H, 2023. Regulation of erythropoiesis: emerging concepts and therapeutic implications. Hematology. 28(1):2250645. Doi: 10.1080/16078454.2023.2250645.

35. Li Q, WongYL, HuangQ, et al, 2014. Structural insight into the transmembrane domain and the juxtamembrane region of the erythropoietin receptor in micelles. Biophys J, 107(10), Pages 2325-2336. Doi: 10.1016/j.bpj.2014.10.013.

36. Middleton SA, Barbone FP, Johnson DL, et al, 1999. Shared and unique determinants of the erythropoietin (EPO) receptor are important for binding EPO and EPO mimetic peptide. J Biol Chem. 274(20), Pages 14163-14169. Doi: 10.1074/jbc.274.20.14163.

37. He Q, Han C, Li G, et al, 2020. In silico design novel (5-imidazol-2-yl-4-phenylpyrimidin-2-yl) [2-(2-pyridylamino) ethyl] amine derivatives as inhibitors for glycogen synthase kinase 3 based on 3D-QSAR, molecular docking and molecular dynamics simulation. Comput Biol Chem. 88, Pages 107328. Doi: /10.1016/j.compbiolchem.2020.107328.

38. Saha J, Choudhuri S, Choudhuri D, 2017. Effect of subchronic exposure to chromium on hematological and biochemical parameters of male albino rat. Asian J Pharm Clin Res. 10(5), Pages 345-348. Doi: 10.22159/ajpcr.2017.v10i5.17468.