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INSILICO DOCKING STUDIES OF VARIOUS COMPOUNDS OF *Euphorbia hirta L.*, PLANT ON FERRITIN, OXYHEMOGLOBIN, HEPCIDIN, AND TRANSFERRIN RECEPTORS

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ABSTRACT

Anemia is characterized as having a lower hemoglobin (Hb) level than normal. Iron supplements are currently prescribed as a medicine for treating anemia. As iron supplements have more side effects such as CNS toxicity and cancer herbal medications are majorly preferred for the treatment of the disease. *Euphorbia hirta L.*, is a plant that is majorly available worldwide and helps treat various diseases. The current research aimed to study the docking analysis of various compounds of the plant *Euphorbia hirta L.*, on receptors such as Ferritin, Oxyhemoglobin, Transferrin, and Hepcidin and to evaluate the results obtained using PyRx software. Alongside docking, Toxicity and ADME analysis is done using Protox 2 and the Swiss ADME website. The ADME results showed that except for camphol, and phenylhydrazine there is no BBB penetration for any compound. The docking result showed that on receptor Ferritin, camphol has the highest binding score of -4.6. For protein Oxyhemoglobin, phenylhydrazine has the highest score of -5.3. For protein Hepcidin -4.7 is the highest score for phenylhydrazine and citronellol. For protein transferrin, citronellol has the highest score of -4.4. It is hypothesized from the current docking research that the chemical compounds of the *Euphorbia hirta L.*, plant have an anti-anemic effect on various individuals and it can also be used in designing new drugs used for anemic therapy.

Keywords: Molecular docking; Anemia; Ferritin; Oxyhemoglobin; Transferrin; Hepcidin; *Euphorbia hirta*

INTRODUCTION

According to the WHO, anemia is defined as having a hemoglobin (Hb) count under Thirteen grams per deciliter in men beyond their teenage years of fifteen, a little over twelve grams per deciliter in non-pregnant women past the maturity of fifteen, and no higher than Eleven grams per deciliter in expectant mothers**(Goddard *et al.*, 2011)**. Higher mortality and hospitalization rates may be related to anemia. Weakness, lack of energy, difficulty paying attention, and low performance at work are symptoms that most likely emanate from failing tissue oxygen delivery. **(Kassebaum, 2015)** A Recent WHO review disclosed that 39.8 percent of babies aged six months to sixty-nine months and 29.9 percent of women aged from fifteen to fifty-nine dealt with anemia**. (Khan N *et al.*, 2023)**. The causes of anemia are divided into two categories; non-nutritional (due to factors like infection, inflammation, blood loss, or genetic disorders) and nutrition-specific (due to factors like iron, vitamin A, riboflavin vitamin B12, or folate deficiencies). **(Williams *et al.*, 2023)**. Iron-rich foods and alterations to their diet are frequently used techniques for the medical care of anemia**(Sheth *et al.*, 2021).**A long-used curative herb in ancient medicine is *Euphorbia hirta L.*, which is uncomplicated to locate this species of shrub in its natural habitat, which includes yards, surfaces of roads, landscaped areas, agricultural land, and a multitude of other locations**(Marina Silalahi, 2021)**. The petite yearly plant *Euphorbia hirta L.*, a species of the Euphorbiaceae family, is commonly encountered in tropical regions. It broadens up to eighty centimeters tall, is normally straight, and has a thin stem, however, it occasionally lies over**. (Yuet Ping *et al.*, 2013)**. A current empirical study has revealed that *Euphorbia hirta L.*, and its key constituents have a variety of medicinal properties, including astringent, antimicrobial, antiseptic, antidiarrheal, tranquilizer, anxiolytic, pain reliever, and fever-reducing actions **(Linfang *et al.*, 2012)**. The herb from its foliage and blossoms has significant amounts of flavonoids, and alkaloids as well as terpenoids, saponins, tannins, and These sections of the plant can be utilized as significant sources of plant chemicals and antibacterial action because they contain carbohydrates in modest amounts **(Ahmad *et al.*, 2017)**. The aforementioned chemical compounds were scrutinized using the PyRx technique on the docking study of human ferritin, transferrin, hepcidin, and oxyhemoglobin. Furthermore, absorption, bioactivity score, toxicity assessments, distribution, metabolism, and excretion (ADME), and ProTox II and Swiss ADME methodologies were used to study these topics **(Kumaraswamy *et al.*, 2023).**

MATERIALS AND METHODS

Selection of proteins

Three-dimensional structures of the proteins such as Ferritin [PDB ID:1GWG] (resolution of 2.01Å), Transferrin [PDB ID: 6WB6] (resolution of 2.05Å), Hepcidin [PDB ID: 3HOT] (resolution of 3.25Å), and Oxyhemoglobin [PDB ID: 1HHO] (resolution of 2.1Å) were obtained using Protein Data Bank (PDB)**(Angmo *et al.*, 2017).**

Selection of ligands

The several ligands such as Afzelin (PubChem CID: 5316673), Camphol (PubChem CID: 6552009), Caryophyllene (PubChem CID: 5281515), Citronellol (PubChem CID: 8842), Gallic acid (PubChem CID: 370), Gamma tocopherol (PubChem CID: 92729), Kaempferol (PubChem CID: 5280863), Myricetin (PubChem CID: 5281672), Protocatechuic acid (PubChem CID: 72), Quercetin (PubChem CID: 5280343), Quercitrin (PubChem CID: 5280459), Rutin (PubChem CID: 5280805), Stigmasterol (PubChem CID: 5280794), Folic acid (PubChem CID: 135398658), and Pheylhydrazine (PubChem CID: 7516) were acquired from PubChem Website(**Kundu *et al.*, 2023).**
The chosen chemical elements are primarily from the Flavonoids (Afzelin, Quercetin, Myricetin, Rutin, and Quercitrin) group, they may have antioxidant effects and be used to cure a variety of ailments, according to findings from the LCMS of the *Euphorbia hirta* leaf. And the remaining chemical constituents are present in sufficient amount in the leaf such as triterpenoids (camphol, caryophyllene) Phenolic acids (gallic acid, protocatechuic acid) and Tocopherols (gamma tocopherol)

Molecular Docking

The docking was executed with the help of the PyRx software version 0.8. Post docking the auto dock preferences were extracted in PDBQT format for the object being docked and binding site. The executed docking was visualized using Biovia software. **(Yotriana *et al.*, 2018).**

Canonical Smiles

Utilizing the Pub Chem internet portal, it can be acquired **(Kumaraswamy *et al.*, 2023).**

ADME studies

Utilizing the Swiss ADME computation approach, chosen ligands underwent an Absorption, Distribution, Metabolism, and Excretion (ADME) study **(Andyni RS *et al.*, 2023).**

Toxicity studies

With the assistance of the Pro Tox II internet portal, the binding substances had their toxicity examined. **(Malhotra, 2022).**

RESULTS AND DISCUSSION

Ferritin receptor

An iron-storing molecule found in the two categories of organisms is called ferritin. It is made up of a protein exterior made up of 24 segments, and when the substance is completed, its mass as a whole is roughly fifty thousand Daltons **(Worwood M, 1990)**. Ferritin's three-dimensional design is displayed in (Fig-1).

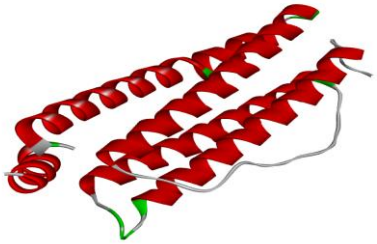


Figure 1 3-dimensional structure of Ferritin

Docking analysis of Ferritin:

The binding energy of the protein ferritin has been found and the resulting scores obtained are -4.3 as the highest score for camphol and -7.6 as the lowest score for the compound folic acid followed by quercetin (-7.5) kaempferol (-7.2), myricetin (-6.9), rutin (-6.8), quercitrin (-6.7), afzelin (-6.6), caryophyllene (-6.3), gallic acid (-5.2), gamma-tocopherol (-5.2), citronellol (-4.6), and phenylhydrazine (-4.4). The smaller the compound's bonding attraction with a specific target, the higher its binding number. The amino acid bonds such as Ala 98, Arg 25, Leu 22, Ser 85, Val 10, Asp 87, Trp 89, Tyr 28, Arg 59, Leu 31, Leu 81, Tyr 28, Ala 98, Leu 102, Arg 39, Phe 35, Arg 39, Asp 40, Ser 32, Trp 89, Arg 18, Arg 25, Leu 102, Ser 85, Ser 105, Thr 29, Arg 39, Asp 38, Ser 32, Arg 39, Gln 86, Lys 83, Phe 35, Pro 84, Ser 32, Ala 55, Arg 52, Leu 24, Leu 31, Leu 81, Tyr 28 Arg 39, Asp 87, Phe 35, Ser 32, Tyr 36, Ala 98, Arg 25, Asp 94, Gln 82, Gln 86, Ser 105, and Thr 25 with Ala 98, Arg 25, Ser 32, Ser 85 as the common chains of all the compounds of the plants of *Euphorbia hirta L.*, that are tabulated in (Table 1).

Table 1 Docking scores of the protein Ferritin and their amino acid bonds

Protein	Chemical compounds	Binding Scores	Amino acid bonds
Ferritin	Afzelin	-6.6	Ala 98, Arg 25, Leu 22, Ser 85, Val 101
	Camphol	-4.3	Asp 87, Trp 89
	Caryophyllene	-6.3	Tyr 28
	Citronellol	-4.6	Arg 59, Leu 31, Leu 81, Tyr 28
	Gallic acid	-5.2	Ala 98, Leu 102
	Gamma tocopherol	-5.2	Arg 39, Phe 35
	Kaempferol	-7.2	Arg 39, Asp 40, Ser 32, Trp 89
	Myrecitin	-6.9	Arg 18, Arg 25, Leu 102, Ser 85, Ser 105, Thr 29
	Quercetin	-7.5	Arg 39, Asp 38, Ser 32
	Quercitrin	-6.7	Arg 39, Asp 38, Asp 87, Ser 32
	Rutin	-6.8	Arg 39, Gln 86, Lys 83, Phe 35, Pro 84, Ser 32
	Stigmasterol	-7.3	Ala 55, Arg 52, Leu 24, Leu 31, Leu 81, Tyr 28
	Phenylhydrazine	-4.4	Arg 39, Asp 87, Phe 35, Ser 32, Tyr 36
	Folic acid	-7.6	Ala 98, Arg 25, Asp 94, Gln 82, Gln 86, Ser 105, Thr 25

2D and 3D structures

The bound molecules' 2D and 3D shapes were derived using Biovia Discovery Studio version 3.0. they can help identify the interactions of the protein with ligands and they are also helpful in providing the name of the bond that is interacted with the compound. The various 2-dimensional and 3-dimensional structures of various compounds with ferritin protein are listed in (Fig-2) and (Fig-3) which show the interaction of the compounds and the type of bonds that are attached to the compound.

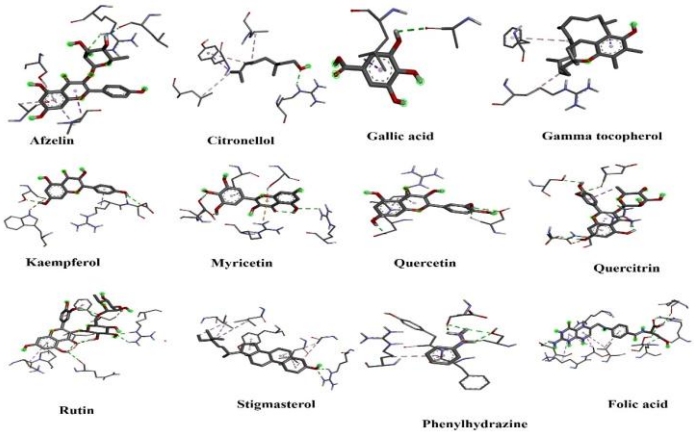


Figure 2 3-dimensional structure of the interaction of compounds with Ferritin

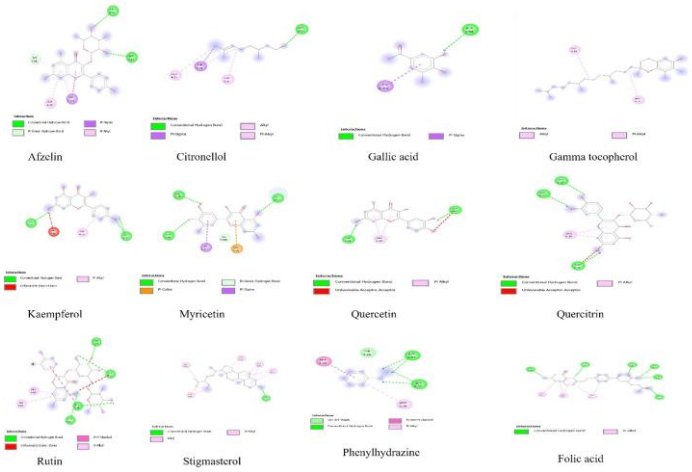


Figure 3 2-dimensional structures of interaction of the compounds with Ferritin

Oxyhemoglobin receptor

Whenever oxygen binds to hemoglobin during circulatory transit, oxyhemoglobin (HbO) is created. It was recently determined that Hb's active O2-binding mostly correlates with changes in its fourth-generation structures, which are discovered to have low and high O2-affinities, specifically, as T(deoxy)- and R(oxy)-quaternary structures**(Ray *et al.*, 2016).** Oxyhemoglobin's three-dimensional shape is depicted in (Fig-4).

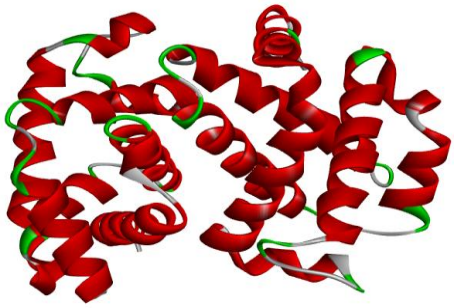


Figure 4 3-dimensional structure of Oxyhemoglobin

Docking analysis of Oxyhemoglobin:

The binding energy of the protein oxyhemoglobin has been found and the resulting scores obtained are -5.3 as the highest score for phenylhydrazine and -8.6 as the

lowest score for the compounds quercetin and rutin followed by kaempferol (-8.5), myricetin (-8.4), afzelin (-8.3), quercitrin (-7.9), stigmasterol (-7.9), folic acid (-7.6), caryophyllene (-6.3), camphol (-5.9), citronellol (-5.9), gallic acid (-5.7), and gamma-tocopherol (-5.5). The smaller the compound's bonding attraction with a specific target, the higher its binding number. The amino acid bonds such as Ala 130, Asp 126, Lys 99, Tyr 35, Val 98, Ala 135, His 58, His 87, Leu 91, Phe 43, Tyr 42, Val 93, His 87, Phe 43, Val 93, Tyr 42, His 63, Leu 91, Leu 96, Phe 41, Val 98, His 87, Leu 101, Val 62, Val 93, Val132, Ser 102, Asn 97, His 87, Leu 101, Leu 129, Met 32, Val 62, Val 93, Val 132, Ala 130, Asp 126, Lys 99, Phe 98, Pro 95, Ser 102, Ala 130, Arg 141, Asp 126, Lys 99, Ser 133, Thr 134, Asp 126, Ala 130, Lys 99, Pro 95, Ser 131, Ala 135, His 103, Leu 100, Leu 105, Lys 99, Lys 132, Phe 36, Pro 37, Asn 102, Phe 41, Phe 42, Val 98, Ala 135, Arg 104, Asn 108, Asp 126, Gln 131, Lys 99, Phe 36, Ser 102, Ser 133, and Tyr 35 with Ala 130, Ala 135, His 87, Leu 99, Leu 101, Lys 99, Phe 43, Pro 95, Val 93, and Val 98 as the common chains of all the compounds of the plants of *Euphorbia hirta L.*, that are tabulated in (Table 2).

Table 2 Docking scores of the protein Oxyhemoglobin and their amino acid bonds

Protein	Chemical compounds	Binding Scores	Amino acid bonds
Oxyhemoglobin	Afzelin	-8.3	Ala 130, Asp 126, Lys 99, Tyr 35
	Camphol	-5.9	Val 98
	Caryophyllene	-6.3	Ala 135
	Citronellol	-5.9	His 58, His 87, Leu 91, Phe 43, Tyr 42, Val 93
	Gallic acid	-5.7	His 87, Phe 43, Val 93, Tyr 42
	Gamma tocopherol	-5.5	His 63, Leu 91, Leu 96, Phe 41, Val 98
	Kaempferol	-8.5	His 87, Leu 101, Val 62, Val 93, Val132, Ser 102
	Myrecitin	-8.4	Asn 97, His 87, Leu 101, Leu 129, Met 32, Val 62, Val 93, Val 132
	Quercetin	-8.6	Ala 130, Asp 126, Lys 99, Phe 98, Pro 95, Ser 102
	Quercitrin	-7.9	Ala 130, Arg 141, Asp 126, Lys 99, Ser 133, Thr 134
	Rutin	-8.6	Asp 126, Ala 130, Lys 99, Pro 95, Ser 131
	Stigmasterol	-7.9	Ala 135, His 103, Leu 100, Leu 105, Lys 99, Lys 132, Phe 36, Pro 37
	Phenylhydrazine	-5.3	Asn 102, Phe 41, Phe 42, Val 98
	Folic acid	-7.6	Ala 135, Arg 104, Asn 108, Asp 126, Gln 131, Lys 99, Phe 36, Ser 102, Ser 133, Tyr 35

2D and 3D structures

The bound molecules' 2D and 3D shapes were derived using Biovia Discovery Studio version 3.0. They can help identify the interactions of the protein with ligands and they are also helpful in providing the name of the bond that is interacted with the compound. The various 2-dimensional and 3-dimensional structures of various compounds with oxyhemoglobin protein are listed in (Fig-5) and (Fig-6) below showing the interaction of the compounds and the type of bonds that are attached to the compound.

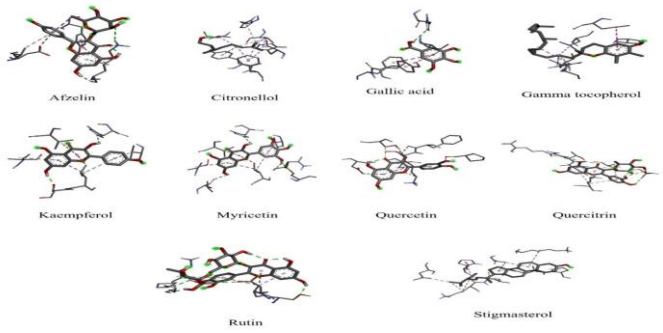


Figure 5 3-dimensional structure of the interaction of compounds with Oxyhemoglobin.

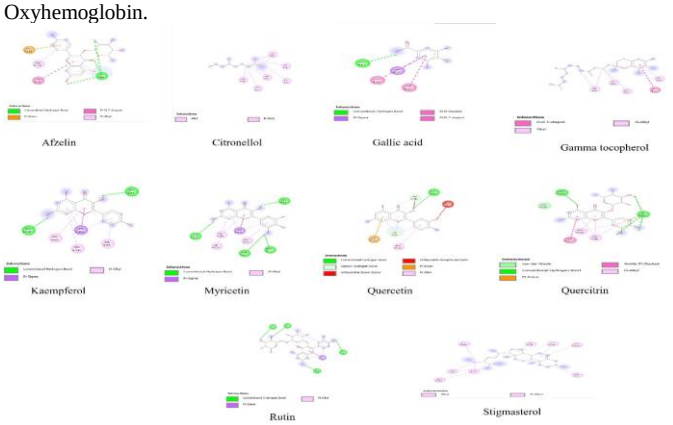


Figure 6 2-dimensional structures of interaction of the compounds with Oxyhemoglobin.

Transferrin receptor

Transferrin receptors are glycoproteins attached to the skin of cells that act as checkpoints to control the amount of iron absorbed by cells from transferrin, an amino acid in plasma that carries iron throughout the body **(Ponka & Lok, 1999)**. Transferrin's three-dimensional shape is depicted in (Fig-7).

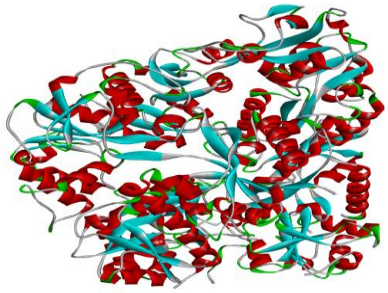


Figure 7: 3-dimensional structure of Transferrin

Docking analysis

The binding energy of the protein transferrin has been found and the resulting scores obtained are -4.4 as the highest score for citronellol and -9.2 as the lowest score for the compounds rutin followed by afzelin (-8.9), folic acid (-8.7), myricetin (-8.6), quercetin (-8.4), quercitrin (-8.4), kaempferol (-8.1), stigmasterol (-8.1), caryophyllene (-6.1), gamma-tocopherol (-6.1), camphol (-5.9), gallic acid (-5.9), and phenylhydrazine (-5.4). The smaller the compound's bonding attraction with a specific target, the higher its binding number. The amino acid bonds such as Asp 527, Asp 548, Glu 535, His 551, Lys 532, Pro 503, Ser 462, Ser 463, Ser 528, Trp 531, Ala 530, His 461, Ser 528, Trp 531, Phe 374, Val 371, Arg 89, Glu 82, Ile 224, Ile 318, Lys 317, Ala 530, Asp 527, Asp 548, Ser 528, Ser 529, Trp 531, Asp 527, Asp 548, Ser 463, Ser 468, Trp 531, Asp 548, His 551, Ser 462, Ser 468, Ser 528, Trp 531, Asp 527, Asp 548, Glu 535, His 551, Pro 503, Ser 462, Ser 463, Trp 531, Asp 409, Asp 527, Asp 548, Lys 532, Phe 467, Pro 584, Ser 465, Ser 545, Trp 531, Ala 635, Phe 552, Tyr 429, Asp 527, Pro 503, Ser 528, Asp 527, Ser 468, Ser 528, Thr 466, Trp 531 with most of the chains as the common chains of all the compounds of the plants of *Euphorbia hirta L.*, that are tabulated in (Table 3).

Table 3 Docking scores of the protein Transferrin and their amino acid bonds

Protein	Chemical compounds	Binding Scores	Amino acid bonds
Transferrin	Afzelin	-8.9	Asp 527, Asp 548, Glu 535, His 551, Lys 532, Pro 503, Ser 462, Ser 463, Ser 528, Trp 531,
	Camphol	-5.9	Ala 530, His 461, Ser 528, Trp 531
	Caryophyllene	-6.1	Phe 374, Val 371
	Citronellol	-4.4	Arg 89, Glu 82, Ile 224, Ile 318, Lys 317
	Gallic acid	-5.9	Ala 530, Asp 527, Asp 548, Ser 528, Ser 529, Trp 531
	Gamma tocopherol	-6.1	Asp 527, Lys 532, Phe 427, Pro 584, Trp 531
	Kaempferol	-8.1	Asp 527, Asp 548, His 551, Ser 462, Ser 468, Ser 529, Trp 531
	Myrecitin	-8.6	Asp 527, Asp 548, Ser 463, Ser 468, Trp 531
	Quercetin	-8.4	Asp 548, His 551, Ser 462, Ser 468, Ser 528, Trp 531
	Quercitrin	-8.4	Asp 527, Asp 548, Glu 535, His 551, Pro 503, Ser 462, Ser 463, Trp 531
	Rutin	-9.2	Asp 409, Asp 527, Asp 548, Lys 532, Phe 467, Pro 584, Ser 465, Ser 545, Trp 531
	Stigmasterol	-8.1	Ala 635, Phe 552, Tyr 429
	Phenylhydrazine	-5.4	Asp 527, Pro 503, Ser 528
	Folic acid	-8.7	Asp 527, Ser 468, Ser 528, Thr 466, Trp 531

2D and 3D structures

The various 2-dimensional and 3-dimensional structures of various compounds with transferrin protein are listed in (Fig-8) and (Fig-9) below that show the interaction of the compounds and the type of bonds that are attached to the compound.

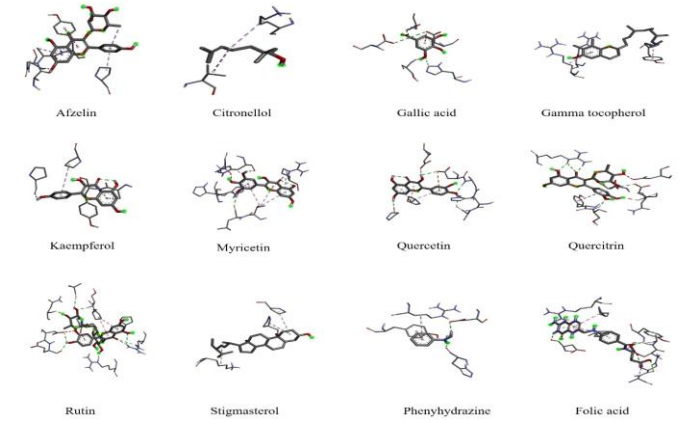


Figure 8 3-dimensional structure of the interaction of compounds with Transferrin

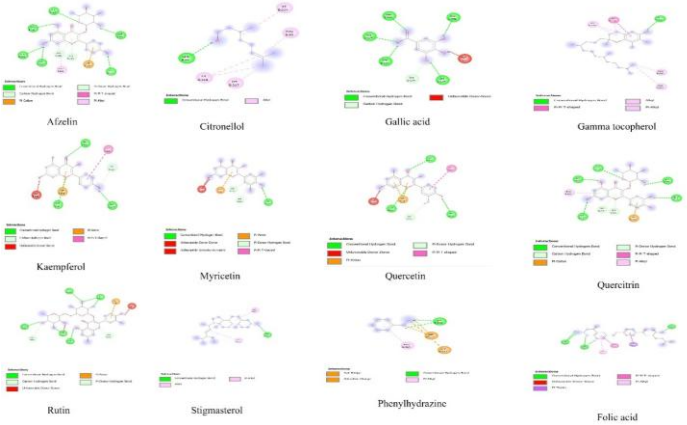


Figure 9 2-dimensional structures of interaction of the compounds with Transferrin

Hepcidin receptor

Hepcidin, a hormonal peptide consisting of 25 peptides, prevents iron from descending the blood vessel segment from each of the main routes of iron: the duodenum's swallowing of food, cells discharge of regenerated iron, and liver discharge of retained iron.**(Ganz & Nemeth, 2012)** Hepcidin's three-dimensional shape is depicted in (Fig-10).

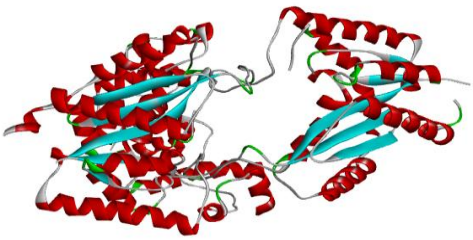


Figure 10: 3-dimensional structure of Hepcidin

Docking analysis

The binding energy of the protein hepcidin has been found and the resulting scores obtained are -4.7 as the highest score for phenylhydrazine and citronellol and -9.3 as the lowest score for the compounds rutin followed by quercetin (-8.2), afzelin (-7.9), folic acid (-7.9), stigmasterol (-7.8), myricetin (-7.7), quercetin (-7.4), kaempferol (-6.9), gamma-tocopherol (-5.8), camphol (-5.3), caryophyllene (-5.2), and gallic acid (-5.2). The smaller the compound's bonding attraction with a specific target, the higher its binding number. The amino acid bonds such as Ala 274, Ala 275, Asn 250, Pro 278, Tyr 276, Ala 182, Val 120, Pro 278, Lys 342, Val 120, Asp 156, Asp 249, Asp 284, His 273, Ser 277, Arg 183, Arg 186, Tyr 276, Arg 260, Asn 250, His 248, His 254, His 273, Leu 271, Val 270, Ala 274, Asn 250, Asp 156, Asp 249, His 273, Pro 121, Pro 278, Arg 186, Asn 250, Asp 156, Asp 249, Asp 284, Ser 277, Tyr 276, Ala 274, Arg 183, Arg 186, Asn 250, Asp 156, Asp 284, Glu 157, Pro 121, Pro 278, Ser 277, Pro 121, Pro 278, Lys 158, Arg 234, Asp 198, His 150, Tyr 237, Ala 274, Ala 251, Arg 183, Asn 250, Asp 284, His 273, Pro 121, Pro 278, and Tyr 276 with Ala 274, Asn 250, Asp 156, His 273, Pro 278, Tyr 276, and Val 120 as the common chains of all the compounds of the plants of *Euphorbia hirta L.*, that are tabulated in (Table 4).

Table 4 Docking scores of the protein Hepcidin and their amino acid bonds

Protein	Chemical compounds	Binding Scores	Amino acid bonds
Hepcidin	Afzelin	-7.9	Ala 274, Ala 275, Asn 250, Pro 278, Tyr 276
	Camphol	-5.3	Ala 182, Val 120
	Caryophyllene	-5.2	Pro 278
	Citronellol	-4.7	Lys 342, Val 120
	Gallic acid	-5.2	Asp 156, Asp 249, Asp 284, His 273, Ser 277
	Gamma tocopherol	-5.8	Arg 183, Arg 186, Tyr 276
	Kaempferol	-6.9	Ala 275, Pro 121, Pro 278, Tyr 276
	Myrecitin	-7.7	Arg 260, Asn 250, His 248, His 254, His 273, Leu 271, Val 270
	Quercetin	-7.4	Ala 274, Asn 250, Asp 156, Asp 249, His 273, Pro 121, Pro 278
	Quercitrin	-8.2	Arg 186, Asn 250, Asp 156, Asp 249, Asp 284, Ser 277, Tyr 276
	Rutin	-9.3	Ala 274, Arg 183, Arg 186, Asn 250, Asp 156, Asp 284, Glu 157, Pro 121, Pro 278, Ser 277
	Stigmasterol	-7.8	Pro 121, Pro 278, Lys 158
	Phenylhydrazine	-4.7	Arg 234, Asp 198, His 150, Tyr 237
	Folic acid	-7.9	Ala 274, Ala 251, Arg 183. Asn 250, Asp 284, His 273, Pro 121, Pro 278, Tyr 276

2D and 3D structures

The bound molecules' 2D and 3D shapes were derived using Biovia Discovery Studio version 3.0. They can help identify the interactions of the protein with ligands and they are also helpful in providing the name of the bond that is interacted with the compound. The various 2-dimensional and 3-dimensional structures of various compounds with hepcidin protein are listed as figures below in (Fig-11)

and (Fig-12) which show the interaction of the compounds and the type of bonds that are attached to the compound.

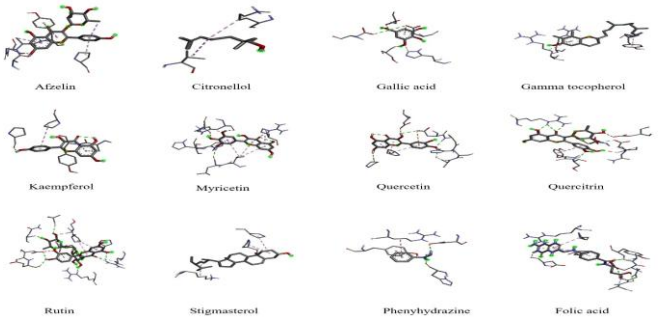


Figure 11 3-dimensional structure of the interaction of compounds with Hepcidin

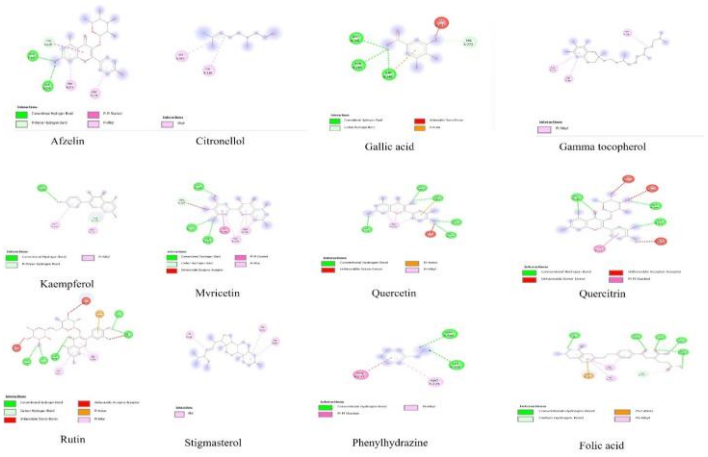
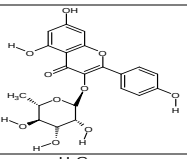
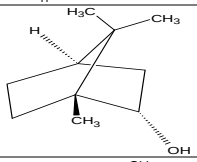
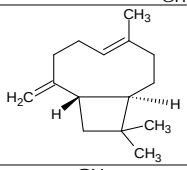
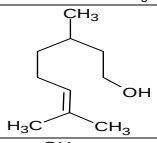
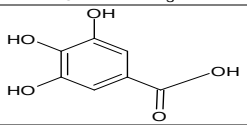
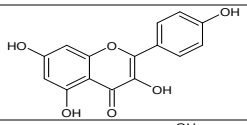
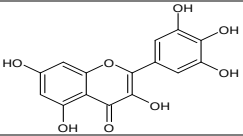
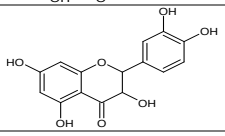
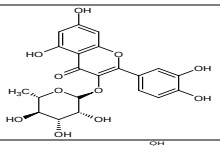
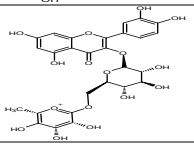
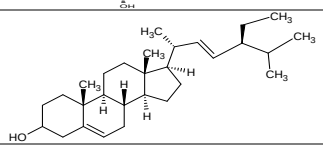
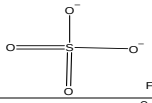
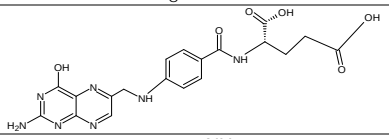
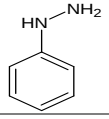


Figure 12 2-dimensional structures of interaction of the compounds with Hepcidin

Ligands: The ligands used in this study are obtained from the PubChem website and the structures, molecular formula, and molecular weight of the compounds are listed in (Table 5). The structures of the compounds were drawn using Chemsketch software **(Ashande *et al.*, 2023).**

Table 5 Molecular Structure, Weight, and Structure of Ligands

Name of the ligands	Molecular formula	Molecular weight (g/mol)	Structures
Afzelin	$C_{21}H_{20}O_{10}$	432.4	
Camphol	$C_{10}H_{18}O$	154.25	
Caryophyllene	$C_{15}H_{24}$	204.35	
Citronellol	$C_{10}H_{20}O$	156.26	
Gallic acid	$C_7H_6O_5$	170.12	
Gamma tocopherol	$C_{28}H_{48}O_2$	416.7	
Kaempferol	$C_{15}H_{10}O_6$	286.24	
Myrecitin	$C_{15}H_{10}O_8$	318.23	
Quercetin	$C_{15}H_{10}O_7$	302.23	

Quercitrin	$C_{21}H_{20}O_{11}$	448.4	
Rutin	$C_{27}H_{30}O_{16}$	610.5	
Stigmasterol	$C_{29}H_{48}O$	412.7	
Ferrous sulfate	FeO_4S	151.91	
Folic acid	$C_{19}H_{19}N_7O_6$	441.4	
Phenylhydrazine	$C_6H_8N_2$	108.14	

Canonical Smiles

The PubChem data of the phytonutrients of *Euphorbia hirta L.*, such as compound CID and canonical Smiles were listed in (Table 6).

Table 6 Simplified Molecular Input Line Entry Specification (smiles)

S.NO	Ligands	Compound CID	Canonical Smiles
1.	Afzelin	5326673	CC1C(C(C(C(O1)OC2=C(OC3=CC(=CC(=C3C2=O)O)O)C4=CC=C(C=C4)O)O)O)O
2.	Camphol	6552009	CC1(C2CCC1(C(C2)O)C)C
3.	Caryophyllene	5281515	CC1=CCCC(=C)C2CC(C2CC1)(C)C
4.	Citronellol	8842	CC(CCC=C(C)C)CCO
5.	Gallic acid	370	C1=C(C=C(C(=C1O)O)O)C(=O)O
6.	Gamma tocopherol	92729	CC1=C(C=C2CCC(OC2=C1C)(C)CCCC(C)CCCC(C)CCCC(C)C)O
7.	Kaempferol	5280863	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O
8.	Myricetin	5281672	C1=C(C=C(C(=C1O)O)O)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O
9.	Quercetin	5280343	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O
10.	Quercitrin	5280459	CC1C(C(C(C(O1)OC2=C(OC3=CC(=CC(=C3C2=O)O)O)C4=CC(=C(C=C4)O)O)O)O)O
11.	Rutin	5280805	CC1C(C(C(C(O1)OCC2C(C(C(O2)OC3=C(OC4=CC(=CC(=C4C3=O)O)O)C5=CC(=C(C=C5)O)O)O)O)O)O)O
12.	Stigmasterol	5280794	CCC(C=CC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C(C)C
13.	Phenylhydrazine	7516	C1=CC=C(C=C1)NN
14.	Folic acid	135398658	C1=CC(=CC=C1C(=O)NC(CCC(=O)O)C(=O)O)NCC2=CN=C3C(=N2)C(=O)NC(=N3)N
15.	Ferrous Sulfate	24393	[O-]S(=O)(=O)[O-].[Fe+2]

ADME analysis

Swiss ADME website is used for the absorption, distribution, metabolism, and elimination data of the compounds of *Euphorbia hirta L.*, and the obtained results are tabulated in (Table 7).

Table 7 Absorption, Distribution, Metabolism, and Excretion (ADME) data of the compounds obtained from Swiss ADME

Ligands	GI[1]	BBB[2]	P-gp[3]	CYP1A2[4]	CYP2C19[4]	CYP2C9[4]	CYP2D6[4]	CYP3A4[4]	Log Kp (cm/s)[5]
Afzelin	L[6]	X	X	X	X	X	X	X	-8.07
Camphol	H[7]	+	X	X	X[8]	X	X	X	-5.31
Caryophyllene	L	X	X	X	+[9]	+	X	X	-4.44
Citronellol	H	+	X	X	X	X	X	X	-4.48
Gallic acid	H	X	X	X	X	X	X	+	-6.84
Gamma tocopherol	L	X	+	X	X	X	X	X	-1.51
Kaempferol	H	X	X	+	X	X	+	+	-6.7
Myrecitin	L	X	X	+	X	X	X	+	-7.4
Quercetin	H	X	X	+	X	X	+	+	-7.05
Quercitrin	L	X	X	X	X	X	X	X	-8.42
Rutin	L	X	+	X	X	X	X	X	-10.26
Stigmasterol	L	X	X	X	X	+	X	X	-2.74
Phenylhydrazine	H	+	X	X	X	X	X	X	-6.07
Folic acid	L	X	X	X	X	X	X	X	-9.76

Note: [1]Gastrointerstinal absorption, [2]Blood-brain barrier permeant, [3]P-gp-P-glycoprotein substrate, [4]CYP- Cytochrome P450 Inhibitors,[5]Log Kp-Skin Permeation (cm/s).
([6]L) – Low, ([7]H)- high, [8](X)- No, [9](+)- Yes.

Toxicity Analysis

Protox 2 is the website that is used for the finding of the toxic potential of the compounds of the plant *Euphorbia hirta L.*, and the obtained data is tabulated in (Table 8).

Table 8 Toxicity analysis of chemical compounds using Protox 2

Compounds	HT[1]	CG[2]	IT[3]	MG[4]	CT[5]	AhR[6]	AR[7]	ARLBD[8]	Aromatase	ER[9]	ER - LB D[10]	PPA R-γ[11]	nrf2/ ARE[12]	HSE[13]	3MMP[14]	p5315 A[15]	ATAD 5[16]	LD50[17]	Toxicity class
Afzelin	-	+	+	-[18]	-	+	-	-	-	-	-	-	-	-	-	-	-	5000	5
Camphol	+[19]	-	+	-	-	-	-	-	+	+	+	-	-	-	-	-	-	1190	4
Caryophyllene	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	5300	5
Citronellol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3450	5
Gallic acid	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2000	4
Gamma tocopherol	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	1190	4
Kaempferol	-	-	-	-	-	+	-	-	+	+	+	-	-	-	+	-	-	3919	5
Myrecitin	-	+	-	+	-	-	-	-	-	-	+	-	-	-	-	+	-	159	3
Quercetin	-	+	-	+	-	+	-	-	-	-	+	+	-	-	+	-	-	159	3
Quercitrin	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5000	5
Rutin	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5000	5
Stigmasterol	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	890	4
Phenylhydrazine	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	175	3
Folic acid	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	135	3

Note: HT[1] - Hepatotoxicity, CG [2]- Carcinogenicity, IT[3] - Immunotoxicity, MG[4] - Mutagenicity, CT[5] - Cytotoxicity. [6]Aryl Hydrocarbon Receptor, [7]Androgen Receptor, [8]Androgen Receptor Ligand Binding Domain, [9]Estrogen Receptor Alpha, [10]Estrogen Receptor Ligand Binding Domain, [11]Peroxisome Proliferator-Activated Receptor Gamma, [12]Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element, [13]Heat shock factor response element, [14]Mitochondrial Membrane Potential, [15]Phosphoprotein (Tumor suppressor), [16]ATPase family AAA domain-containing protein 5, [17]Lethal Dose, [18](-) Inactive,[19](+) Active.

CONCLUSION

A molecular docking study was carried out using PyRx software for the chemical compounds of the plant *Euphorbia hirta L.*, on various receptors such as Ferritin, Oxyhemoglobin, Transferrin, and hepcidin. The docking result showed that on receptor Ferritin, camphol has the highest binding score of -4.6 and folic acid has the lowest binding score of -7.6. For protein Oxyhemoglobin, phenylhydrazine has the highest score of -5.3, and quercetin and rutin have the lowest score of -8.6. For protein Hepcidin -4.7 is the highest score for phenylhydrazine and citronellol and -9.3 is the lowest for compound rutin. For protein transferrin, citronellol has the highest score of -4.4, and rutin has the lowest binding score of -9.2. It is hypothesized from the current docking research that the chemical compounds of the *Euphorbia hirta L.*, plant may have an anti-anemic effect on various individuals and it can also be used in designing new drugs used for anemic therapy. However, further detailed investigation is required for proper drug design for the disease. As the invivo study is not performed in the institution so I couldn't able to attach the data.

Conflict of interest: The authors declare that there is no conflict of interest.

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