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# Inhibitory activity of polyphenolic compound extracted from *P. betel* and *T. aestivum* with hepcidin for Iron deficiency anemia: An *in silico* approach

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Anaemia is a condition that occurs when haemoglobin levels decrease below normal and a lower-than-normal number of healthy red blood cells are produced. A newly identified iron regulator, hepcidin reveals the body's iron status and demand for erythropoiesis to the intestine which modulates intestinal iron absorption. Hepcidin plays a significant role in the iron regulatory system as it naturally binds to ferroportin which is the cellular iron expoter. The hepcidin was docked with bioactive compounds in catechin, chlorogenic acid, *T. aestivum* grass and 2,4 dihydroxy benzaldehyde in *P. betel* leaves. The aim of the current study is to investigate the interaction of bioactive compounds with hepcidin through molecular docking. All three bioactive compounds revealed significant interaction with hepcidin. Catechin showed highest binding energy (−7.1 Kcal/mol) as compared to cholorogenic acid and 2,4 dihydroxy benzaldehyde.

**Keywords**: 2,4-dihydroxybenzaldehyde, Catechin, Chlorogenic acid, Ferroportin, Hepcidin

World Health Organization define anaemia as a condition in which number of red blood cells or the haemoglobin concentration within them is lower than normal. The normal heamoglobin range is 13.2-16.6 g/dL for men, 11.6-15 g/dl for women[1]. According to a report by WHO in 2019, 29.9% of women aged 15-49 years and 39.8% of infants aged 6-59 months suffered from anaemia. Iron deficiency, thalassemia and hemoglobinopathies, folate deficiency, and parasitic illnesses are the most common causes of anaemia (especially malarial and hookworm infections and schistosomiasis)[2]. In developed countries, iron deficiency anaemia is most commonly caused by persistent blood loss, generally from the gastrointestinal tract or uterus. On the contrary dietary iron insufficiency remains the principal cause of iron deficiency anaemia in the undeveloped countries[3].

Iron is an essential micronutrient for life. It conducts vital biological activities such as production of heme-moiety of haemoglobin required for oxygen transportation, repair mechanism, respiratory process, energy metabolism and host defence mechanism[4]. Hepcidin plays significant role in iron metabolism. It is made up of 25 amino acids and has four disulphide linkages[5]. Figure 1 depicts the 2D and 3D structures of hepcidin. Hepcidin is a peptide hormone that is predominantly synthesized by the liver and released into the bloodstream. In the interaction with iron and inflammation, hepcidin production increases and declines in response to erythropoiesis. Hepcidin regulated systemic iron metabolism by interacting with its receptor ferroportin. Ferroportin is a transmembrane iron export protein abundantly expressed on the surface of reticuloendothelial (RE) macrophages and the basolateral membrane of duodenal enterocytes. These two cell types are the primary iron providers to the plasma. RE macrophages recycle iron from senescent erythrocytes, releasing 20-25 mg of iron into the plasma every day. Enterocytes augment additional 1-2 mg of iron to the plasma through the absorption of dietary iron. Hepcidin blocks iron release at both locations by binding to cell-surface ferroportin and inducing internalisation and degradation. It regulates the iron through negative regulatory system. As a result, hepcidin can be seen as a negative regulator of iron absorption and recycling[6-8]. Lack in hepcidin-mediated FPN control, caused by hereditary hemochromatosis leads to iron overload and broad tissue damage in the liver, heart, pancreas, and joints[9-11]. Conversely, excessive hepcidin elevation results in iron-restricted anaemia[12,13].

Plants include natural substances such as polyphenols, flavonoids, taxols, and others that are known to lower the risk of illnesses such as cancer,

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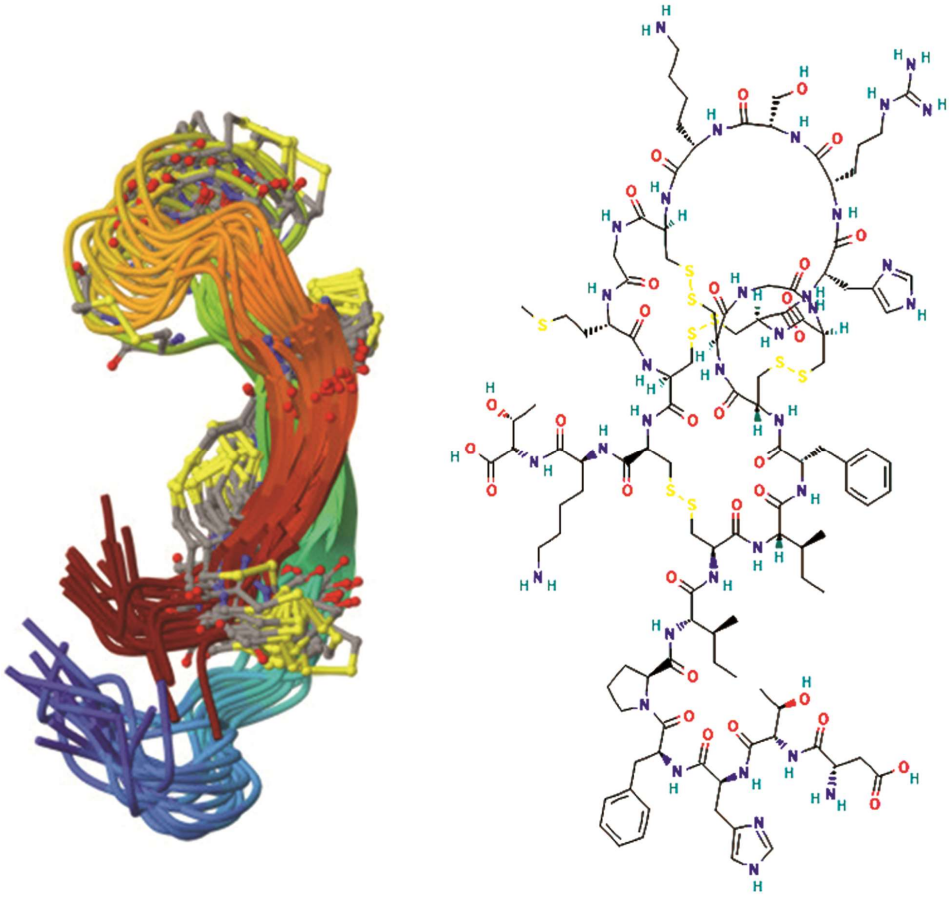


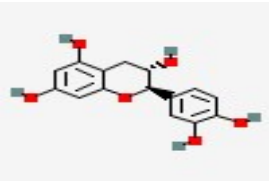
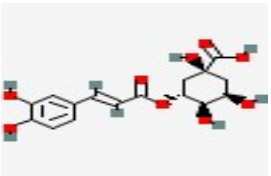
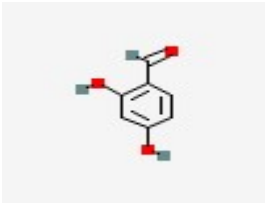
Fig. 1 — 3D and 2D structure of hepcidin

diabetes, and neurological disorders[14,15]. Phenolics, flavonoids, and alkaloids are the secondary metabolites produced by plants as a result of metabolic processes. *Momordica charantia* ethanolic extract contains a variety of phytochemical components such as alkaloids, phenols, and flavonoids. It has anti-psoriatic properties[16]. Various reports indicate the presence of phytochemical compounds such as alkaloids, flavonoids, steroids, phenols, and tannin in the extracts of *Piper betel* leaves & *Triticum aestivum* grass[17,18]. HPLC analysis reveals peaks of catechin, chlorogenic acid, and 2,4-dihydroxy benzaldehyde phenolic compounds[19]. Piper betel leaves have sugar, diastases and high levels of antioxidants like hydroxychavicol, eugenol, ascorbic acid, and beta-carotene[20]. Plant phytochemicals or polyphenolic compounds may regulate oxidation and stress-related diabetes and cardiovascular diseases[21]. In phenyl-hydrazine-induced anaemic rats, butanolic extracts of *T. aestivum* and *P. betel* leaves had a substantial impact[22].

Flavonoids possess high iron chelation ability and are a reliable antioxidant. It can regulate iron metabolism and be used to treat iron overload. Catechins are natural polyphenolic compounds belonging to the flavonoid family. Catechin called flavan-3-ol or flavonol is abundant in fruits, vegetables and plant-based drinks[23]. Chlorogenic acid is a phenolic compound containing antioxidant properties[24]. 2,4 di-hydroxybenzaldehyde also belongs to the flavonoid family[25].

The various range of functional groups of secondary metabolites binds the pathogen at the target site, which is essential for drug discovery. Flavonoids showed inhibitory activity with SARS CoV-2[26]. Molecular docking is one of the most widely used and successful structure-based *in silico* strategies for predicting interactions between molecules and biological targets. It is frequently carried out by first predicting a ligand's molecular orientation within a receptor and then assessing its complementarity using a scoring function[27]. The relevance of binding in molecular docking studies

Table 1 — Molecular properties and structure of bioactive compound

| Sl. No. | Compounds | ID | Molecular weight (g/mol) | Molecular formula | Molecular Structure |
|---|---|---|---|---|---|
| 1 | Catechin | 9064 | 290.27 | C15H14O6 |  |
| 2 | Chlorogenic acid | 1794427 | 354.31 | C16H18O9 |  |
| 3 | 2,4-dihydroxy benzaldehyde | 8768 | 138.12 | C7H6O3 |  |

lies in its ability to provide insights into ligand-protein interactions, elucidate the molecular mechanisms of drug action, guide drug design, and facilitate virtual screening for novel therapeutics. By understanding and optimizing binding interactions, researchers can develop more effective and targeted drugs[28]. Flavonoids regulate iron overload acting as natural drugs for iron homeostasis disorders. High hepcidin level in the serum increases the inhibition of iron absorption, thereby leading to chronic iron deficiency.

The present study aims to identify the inhibitory effect of natural phytochemical compounds present in butanoic extracts *of Piper betel* leaves and *Triticum aestivum* grass with hepcidin by *in silico* approach.

## Materials and Methods

### Data tracking

Three-Dimensional Structural composition of potential protein targets. Hepcidin (https://www.rcsb.org/structure/1m4f) from the protein data bank were downloaded with their respective PDB IDs in a folder. A total of two proteins were retrieved. CASTp11 was used to anticipate the proteins' active sites. Computed Atlas of Surface Topography of proteins. Recent theoretical and algorithmic developments in computational geometry are the foundation of CASTp. It offers a lot of benefits: 1) Analytical identification of pockets and cavities, 2) accurate definition of the border between the bulk solvent and the pocket, 3) rotational invariance of all derived parameters, absence of discretization, dot surface, or grid points. The two-dimensional structure of Catechin, Chlorogenic, 2, 4-Dihydroxy-benzaldehyde were retrieved from PubChem with their respective PubChem CID in a separate ligand folder illustrating their molecular properties and structure (Table 1).

### Molecular Docking

For understanding the proteins and ligands interactions, molecular docking analysis were practiced using the software's PyRx – Python Prescription 0.8 in Windows 10 Home Single Language workstation. The protein and ligand were organized by using AutoDockTools-1.5.7. The proteins [Hepcidin (1m4f)] preparation involves addition of polar hydrogen's, united atom Kollman charge in the virtual environment. The ligands [Catechin, Chlorogenic Acid, 2, 4-Dihydroxybenzaldehyde] were created by adding polar hydrogen's, Gasteiger charge, detect flexible torsions and setting the number of torsions. Gridbox were described based on the size of each protein's active site. Exhaustiveness factor of 10 is given to obtain more consistent docking result. Several conformations were reserved and interactions were analysed manually and best pose with low affinity was selected. Based on the crystal structures of confirmed protein therapeutic targets, investigations of protein-ligand docking were conducted.

The phytochemical compounds of *Piper Betle* & *Triticum aestivum* utilizing the Swiss-ADME software, were simulated screened. (http://www.

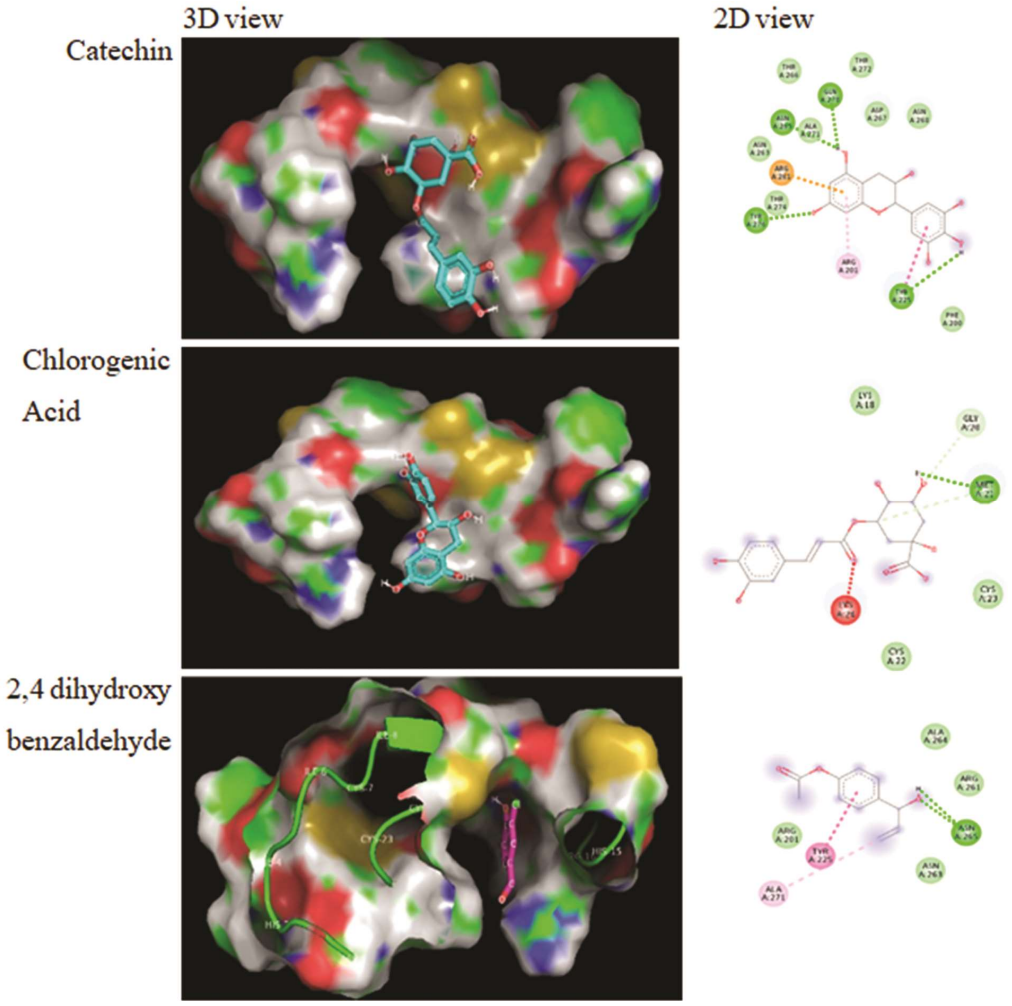



Fig 2 — Two dimension (2D) and three-dimension 3D simulation by molecular docking of Catechin, Chlorogenic acid, 2,4 dihydroxybenzaldehyde with hepcidin

swissadme.ch) to evaluate the molecules using the Lipinski rule and criteria for drug-likeness, such as pharmacokinetic parameters. The investigated compounds are then subjected to molecular docking using Auto Dock Tools (Version 1.5.7) and PyRx - Python Prescription 0.8 in a Windows 10 Home Single Language workstation to properly evaluate their ability to bind to the iron-binding receptor, Transferrin (3qyt), and iron ion trans-membrane transporter inhibitor; signaling receptor, Hepcidin (1m4f).

## Results

The hepcidin protein (1m4f) was docked using AutoDock, Pyrx, Discovery Studio, and PyMol with bioactive compounds present catechin, chlorogenic acid in butanolic extracts of *T. aestivum* grass and 3,4 dihydroxy benzaldehyde in *P. betel* leaves found in HPLC analysis in our previous study. The interaction of three bioactive compound with hepcidin is shown in 2D and 3D view (Fig. 2). In the current study, all three compounds showed a significant inhibitory effect. Catechin showed an unfavourable acceptor-acceptor interaction with hepcidin through the residue of ARG A:16, and LYS A:24. The binding energy of catechin is −7.1 Kcal/mol. The chlorogenic acid had hydrogen bond interaction with ALA A:271, GLN A:270, TYR A:276, TYR A:225. TYR A: 225 also showed the pi-pi interaction. ARG A:261 had pi-cation interaction. The binding energy of −5.3 Kcal/mol. 2, 4-dihydroxy benzaldehyde had H-bond interaction with SER A: 189, TYR A: 188, ARG A: 124 and GLU A:15 residue. Pi-pi T-shaped interaction with PHE A: 186 and pi-sigma interaction with THR A: 181. The binding energy of 2, 4-dihydroxy benzaldehyde is 4.4 Kcal/mol (Table 2).

Table 2 — Molecular docking of catechin, chlorogenic acid, 2,4 dihydroxy benzaldehyde

| Compounds | Pubchem ID | Interaction | Residue | Distance | Binding energy (Kcal/mol) |
|---|---|---|---|---|---|
| Catechin | 9064 | H-bond | ASN A:265 | 25.0 | –7.1 |
| | | Pi-Pi stacked | TYR A:225 | 21.9 | |
| | | Alkyl | ALA A:271 | 19.4 | |
| Chlorogenic acid | 1794427 | H-bond | ASN A:265 | 29.7 | –7 |
| | | H-bond | GLN A:270 | 27.0 | |
| | | H-bond | TYR A:225 | 21.3 | |
| | | H-bond | TYR A:276 | 29.1 | |
| | | Pi-cation | ARG A: 261 | 22.5 | |
| | | Pi-Pi stacked | ARG A: 261 | 25.3 | |
| | | Pi-alkyl | ARG A:201 | 27.7 | |
| 2,4-dihydroxy benzaldehyde | 8768 | H-bond | GLU A:15 | 53.1 | –5.4 |
| | | H-bond | ARG A:124 | 41.5 | |
| | | H-bond | TYR A:188 | 42.2 | |
| | | H-bond | SER A:189 | 57.9 | |
| | | C-H bond | THR A:61 | 37.2 | |
| | | Pi-sigma | THR A:181 | 53.0 | |
| | | Pi-Pi T-shaped | PHE A:186 | 53.2 | |

## Discussion

Anaemia is a major worldwide health issue that mostly affects women and children in both developed and developing countries. Iron deficiency is the most prevalent cause of anaemia worldwide. Iron deficiency results from a disruption in iron metabolism. The disruption of iron homeostasis in the human body is a retaliatory response to increased immunological activity, which results in iron insufficiency for its biological tasks[29]. Hepcidin is a naturally occurring hormone that affects iron metabolism. It is a key regulator of iron absorption and distribution to tissues in humans. Iron problems in humans are caused by hepcidin or FPN imbalance. As a result, iron homeostasis must be strictly regulated. In recent years, several inhibitors targeted at decreasing hepcidin synthesis by binding have been investigated to manage iron homeostasis. Molecular docking is a powerful and useful approach for drug design and discovery that is based on the binding or interaction of molecules. Binding refers to the specific interaction between a ligand and a target molecule through non-covalent interactions[30]. In reported research, this tool provided valuable information that will be useful in drug designing for oral cancer[31] and vaccine production for Human Papilloma Virus[32]. The relevance of binding lies in the ability of polyphenolic compounds to interact with hepcidin and modulate its activity. By binding to hepcidin, these compounds can potentially inhibit its function and restore normal iron metabolism, thereby alleviating iron deficiency anemia. Some hepcidin antagonists have been proven to improve iron homeostasis[30,33-35]. Miraxanthin-V, Liriodenin, and Chitranone are plant-derived compounds with better binding affinity and chemical resemblance to the conventional anticalin described by Yotriana *et al*[36].

Present study showed the interaction of bioactive compounds present in butanolic extracts of *T. aestivum* grass and *P. betel* leaves through docking. In the previous study, three phytochemical compounds *i.e*. catechin from butanolic extracts of *T. aestivum* grass and Chlorogenic acid, 3,4 dihydroxy benzaldehyde from butanolic extracts of *P. betel* leaves were characterized through HPLC analysis[19]. Kumari *et al.,* 2015 and Rekha *et al.,* 2014 reported the presence of main compounds like chavicol, carvacrol, methyl eugenol, isoeugenol, hydroxy chavicol, etc., along with other constituents in *P. betle* leaves[37,38]. Catechin, Chlorogenic acid, 2,4 dihydroxy benzaldehyde were strongly inhibited by hepcidin with −7.1, 7, 5.4 Kcal/mol, respectively, catechin and chlorogenic acid have the highest binding energy. Limanto A *et al*. observed comparable findings in their research of quercetin and rutin, both flavonoid compounds having binding energies of −6.7 Kcal/mol and 8.7 Kcal/mol, respectively[39]. In comparison to these two, 2,4-dihyroxybenzaldehyde had a lower binding energy. The findings in the current study provide light on inhibitory impact of hepcidin.

## Conclusion

The blind docking studies revealed that the bioactive compounds catechin, chlorogenic acid and 2,4-dihydroxybenzaldehyde showed potential inhibition

with the targeted protein hepcidin. Catechin has the highest binding energy among these bioactive chemicals. Binding of bioactive substances to hepcidin may aid in regulation of iron homeostasis.

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**Conflicts of interest**

All authors declare no conflicts of interest.

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