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# Molecular Structure-Affinity Relationship of Flavonoids in Mango Pulp on Binding to Human Serum Albumin

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**Abstract:**

Mango pulp extract has gained growing attention as nutraceutical due to its various pharmacological functions. The polyphenolic phytochemical, flavonoids are considered as one of the main functional components of mango pulp. The structural variation present in flavone compounds such as hydroxylation in different positions and glycoconjugation in flavonoid structure, affect their binding properties with human serum proteins. Human serum proteins such as albumin, alpha fetoprotein, transferrin etc. can act as carrier proteins for flavonoid group of ligands, which can transport the ligands to their target sites. During transportation the binding affinity of the ligands with serum protein albumin can regulate their biological activities. In this study, the presence of four flavonoids in ethanolic extract of mango pulp is confirmed by UV-spectroscopic study. The molecular interaction studies between four flavonoids and human serum albumin are explored by in-silico method in molecular level. The connection between the molecular structures of flavonoids and their binding affinities for human serum albumin (HSA) is compared with the help of molecular docking method. The hydroxylation on 5 and 7 positions of A ring of flavone structure increases the affinities of ligands towards human serum albumin protein. Apart from the hydroxylation on 5 and 7 positions, the glycosylation on 3 position of C ring structure of flavone residue, also causes affinity induction for the same protein. It is revealed from the molecular docking study, that not only hydrogen bonds, but also, non-covalent interactions like, Pi-alkyl, Pi-Pi T shaped and Van der Waals interactions, play important roles in binding of flavonoids present in mango pulp to serum human albumin.

***Keywords:*** *Binding Affinity; Flavonoids in Mango Pulp; Human Serum Albumin; Molecular Docking; Non-Covalent Interactions* Introduction

Throughout the plant kingdom, flavonoids are a vast class of naturally occurring low molecular mass polyphenol compounds that serve a variety of crucial roles, including chelating and antioxidant capacities. In vitro studies have demonstrated that flavonoids possess a broad spectrum of biological and pharmacological properties, such as anti-allergic, anti-inflammatory, antioxidant, antimicrobial, antibacterial, antiviral, and antifungal properties, as well as anti-diarrheal and anti-cancer properties (Fang *et al*.,

2024). They are primarily found in vegetables (Eramma *et al*., 2025), fruits (Maheshwari, Rajawat & Parashar, 2024), as well as in wines, cocoa (Singh, Khedkar & Chandra, 2024), and teas consumed in the human diet.

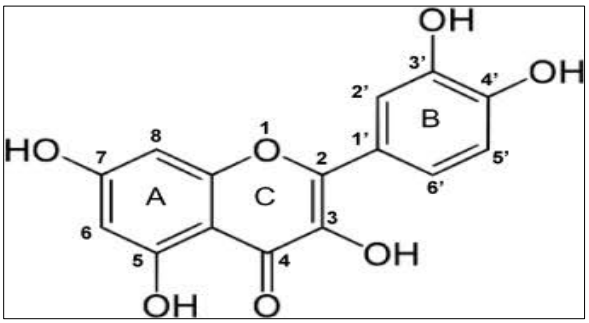


**Figure 1:** General Structure of Flavonoids *(Source: PubChem)*

Flavonoids are a broad class of polyphenolic compounds derived from plants that have a general structure (Figure 1). They are primarily found in fruits (Mehmood, Mehmood & Zulfiqar 2024), vegetables, and medicinal plants and are important in the detoxification process of free radicals. According to reports, quercetin and rutin are used to treat obesity and cardiovascular diseases. They also have antiviral, antibacterial, anti-inflammatory, antioxidant, and radical-scavenging properties. They are also immune-modulator agents and have therapeutic activity in conditions resulting from oxidative stress (Alsaif *et al*., 2020). Flavonoids are anticancer compounds, present in edible mango pulp, considered as nutraceuticals. Four flavonoids e.g. 5 hydroxyflavone (primuletin), 7 hydroxyflavone, 5, 7 dihydroxyflavone (chrysin) and kaempherol-3-glucuronide (Figure 2) are present in ethanolic extract of himsagar mango (Basu, 2024). All four phytochemicals after absorption through epithelial cells of GIT, transported to target cells after binding with albumin protein.

About 7% of the protein in human blood serum is found in the albumin fraction, which makes up two thirds of the total, and the globulin fraction, the remaining third. A big albumin peak and three smaller globulin peaks—the alpha, beta, and gamma globulins—are visible on serum electrophoresis. The single free sulfhydryl (—SH) group present in albumin (molecular weight 68,000) oxidizes to form a disulfide bond (S-S) with the sulfhydryl group (-SH) of another serum albumin molecule, forming a dimer. Serum albumin has an isoelectric point of pH 4.7.

About 60% of human plasma proteins are made up of serum albumin, a biomolecule that is crucial for the distribution and transportation of medications, metabolites, hormones, and other substances. It appears to function as a carrier for specific biological substances in living things (Ghosh & Bhadra, 2024). Serum albumin, a protein that stabilizes other proteins and is found in blood serum at a comparatively high

concentration, serves as a protective colloid. For this reason, information about the ligands' binding mechanism on the available human serum albumin (HAS) binding sites is provided by the spectroscopic, docking, and molecular dynamic simulation methods. Sudlow sites I and II, which are situated in subdomains IIA and IIIA, are the two well-known binding sites that are accessible on HSA (Wang *et al.*, 2020).

The rationale for finding and creating naturally occurring compounds that are pharmacologically active and capable of binding the target plasma proteins has been greatly advanced by the latest developments in computational techniques. In order to demonstrate the biological activities of the natural compounds derived from mangos, computational methods have been employed for analysis (Basu, 2024). In our earlier study, we have identified, kaempherol-3-glucuronide, 5-hydroxy flavone (primuletin), 7-hydroxy flavone, chrysin, as potential therapeutic compounds present in himsagar mango pulp by using both UV visible spectroscopy and computational approaches (Basu, 2024).

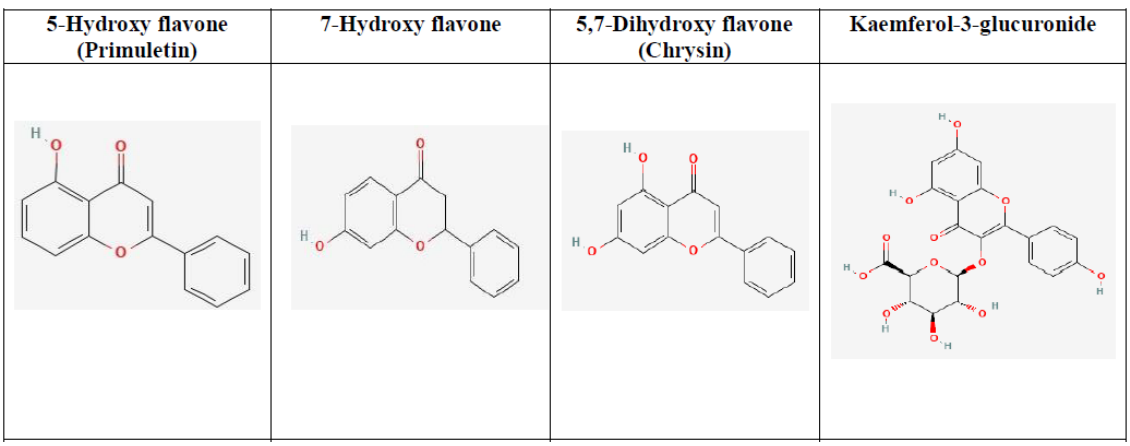


**Figure 2:** Chemical Structures of Four Flavonoids *(Source: PubChem)*

Flavonoids interact with various water-soluble proteins, especially albumins (López-Yerena *et al*., 2020), when they are present in blood. Research on luteolin (Sarmah *et al*., 2020), taxifolin, catechins (Shi *et al*., 2011), galangin, naringenin (Yazdani *et al*., 2022), kaempferol, diosmetin (Zhang, Wang & Pan, 2012), and other flavonoids described this interaction (Xue *et al*., 2021). Dihydrochalcone and bovine serum albumin (BSA) were found to interact spontaneously, under the influence of hydrophobic forces, and with the release of energy (Curvale *et al*, 2012). While apigenin comes into contact with site I of subdomain II of BSA (Zhao *et al*., 2012), morin was found to spontaneously interact with site II of subdomain IIIA of BSA (Hu *et al*., 2012). Fisetin occupied a site similar to that of human serum albumin (HSA) (Singha Roy *et al*., 2012). Hesperetin's binding constant to HSA was $1.941 \times 10^{-4}$ M (Ding *et al*., 2012), while EGCG's binding constant to site I of BSA's subdomain IIA was $6.6 \times 10^{-7}$ M. EC's binding constant to site

II of subdomain IIIA was $10^{-6}$ M. It was proposed that the impact of galloyl groups could explain the observed variation in catechin binding (Pal *et al*., 2012).

## Methodology

### *A. UV-Spectrophotometric screening for determination of Flavonoids present in mango pulp*

The ethanolic sample of himsagar mango pulp was subjected to UV-spectrophotometric screening between 200 and 450 nm. The absorbance and peaks are recorded to order to identify the flavonoids in the mango sample. In order to accurately identify the flavonoids, found in mango pulp extracts, the ethanolic extract is treated with a UV shift reagent, such as NaOAc, and the absorption spectra are examined. During UV spectrometric analysis, various absorption maxima (peaks and shoulders) are observed.

### *B. Molecular docking study of plasma proteins with flavonoids present in mango extract*

A crucial tool in computer-assisted drug design and structural molecular biology is molecular docking. Predicting the predominant binding mode(s) of a ligand with a protein that has a known three-dimensional structure is the aim of ligand-protein docking. Effective docking techniques use a scoring function that appropriately ranks candidate dockings and efficiently searches high-dimensional spaces. Protein-ligand interaction research benefits greatly from the use of docking, which can be used to virtually screen huge libraries of compounds, rank the outcomes, and suggest structural hypotheses about how the ligands bind to the target. Molecular docking techniques have demonstrated how these natural products interact with human serum albumin (Patil & Rohane, 2021; Bugnon *et al*., 2024).

### *C. 2D plot generation of Protein-ligand interaction for human serum albumin and flavonoids*

By using Discovery studio visualizer software (Jejurikar & Rohane, 2021), four 2D interaction plots of human serum albumin and four flavonoids interactions, are studied to elucidate their types of bindings interactions and amino acid residues of human serum albumin which are involved in flavonoids binding.

## Results and Discussion

### *A. UV spectrophotometric absorption curve for mango extract*

With the ethanolic sample of himsagar mango pulp, UV-spectrophotometric screening has been completed within the range of 200-450 nm. The peaks and absorbance are noted and the presence of the four flavonoids has been identified in mango sample.

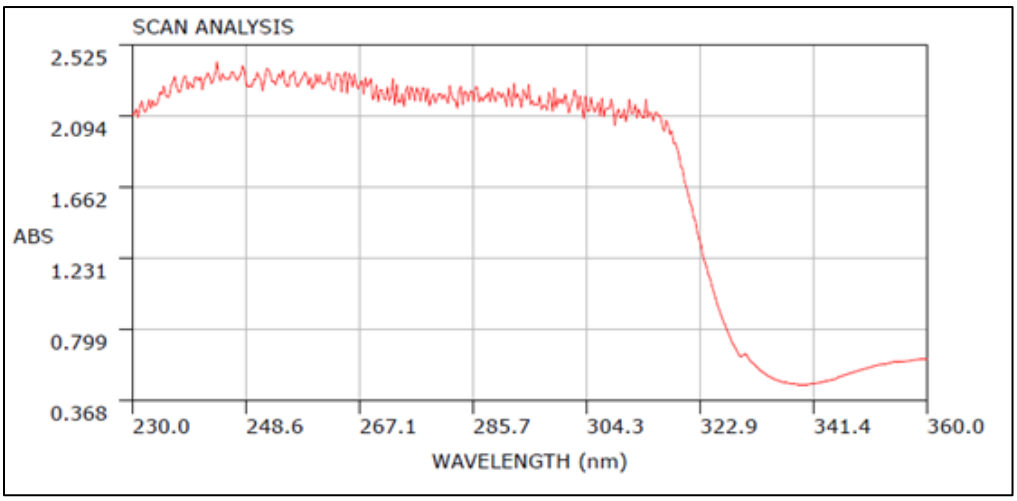


**Figure 3:** UV Spectrophotometric Absorption Curve for Mango Extract *(Source: PubChem)*

### *B. Observation of absorption maxima after addition of the UV shift reagent sodium acetate*

In presence of UV shift reagent such as sodium acetate, a bathochromic shift is observed in in the ethanolic extract, of himsagar mango sample (as shown in Table 1) and the existence of 5-Hydroxyflavone, 7-Hydroxyflavone and 5,7-Dihydroxyflavone in himsagar sample is confirmed.

***Table 1:*** *Absorption Band in Presence of Sodium Acetate*

| Sample | Band Obtained with UV Shift Reagent: Sodium Acetate | Inference |
|---|---|---|
| 5 (Himsagar sample) | 295 (BAND II) | The bathochromic shift from 263 nm to 295 nm confirm the presence of 5-Hydroxyflavone, 7-Hydroxyflavone and 5,7-Dihydroxyflavone |

### *C. Molecular docking study of plasma proteins with flavonoids present in mango extract*

The molecular docking study of human serum albumin (PDB ID 6M4R) with four flavonoids such as 5-Hydroxyflavone, 7-Hydroxyflavone, 5,7-Dihydroxyflavone and kaempherol-3-glucuronide for binding affinity estimation has been conducted. The binding energies, ΔG

(Kcal/mol) and different parameters for binding energy calculations are shown in Table 2.

***Table 2:*** *The Binding Energies, ΔG (kcal/mol) and Different Parameters for Docking Structures*

| Docking Structure | ΔG (Kcal/ mol) | ΔG-electro (Kcal/ mol) | ΔG-vdw (Kcal/ mol) | ΔG-ligand solvent polar (Kcal/ mol) | ΔG-ligand solvent non-polar (Kcal/ mol) | ΔG-protein solvent polar (Kcal/ mol) | ΔG-protein solvent non-polar (Kcal/ mol) | ΔG-component solvent polar (Kcal/ mol) | ΔG-component solvent non-polar (Kcal/mol ) |
|---|---|---|---|---|---|---|---|---|---|
| 1. Albumin _Primuletin (Cluster 0, Element 0) | -6.90 | 0.0 | -30.61 | -5.76 | 5.33 | -4343.73 | 419.991 | -4339.64 | 418.769 |
| 2. Albumin _7-OH flavone (Cluster 13, Element 1) | -6.98 | 0.0 | -40.82 | -7.92 | 5.35 | -4343.73 | 419.991 | -4326.3 | 418.107 |
| 3. Albumin _Chrysin (Cluster 0, Element 4) | -7.05 | 0.0 | -32.98 | -8.32 | 5.84 | -4343.73 | 419.991 | -4340.62 | 419.058 |
| 4. Albumin _Kaempherol-3-glucuronide (Cluster 1, Element 3) | -8.87 | 0.0 | -64.64 | -23.72 | 10.55 | -4343.73 | 419.991 | -4329.68 | 419.203 |

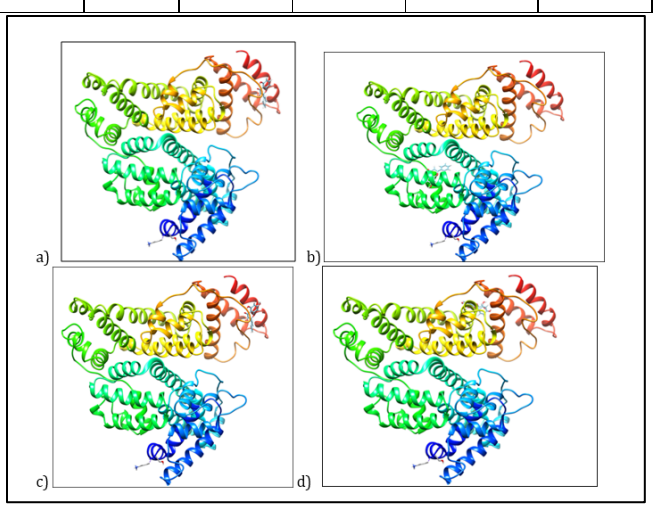


**Figure 4:** Molecular Docking Structures of Human Serum Albumin and Four Flavonoids: A) Molecular Docking Structure of Albumin (Alpha Chain) with Primuletin; B) Molecular Docking Structure of Albumin (Alpha Chain) with 7-Hydroxy Flavones; C) Molecular Docking Structure of Albumin (Alpha Chain) with Chrysin; D) Molecular Docking Structure of Albumin (Alpha Chain) with Kaemferol-3-Glucuronide

Molecular docking study (Bugnon *et al*., 2024) shows that the hydroxylation on 5 and 7 position increases the binding affinities of flavones towards human serum albumin. Glycosylation on position 3 of kaempherol-3-glucuronide also increases the binding affinity for human serum albumin. Different binding sites of human serum albumin with four flavonoids are shown in Figure 4.

### *D. 2D plot generation of Protein-ligand interaction for human serum albumin and flavonoids*

From 2D plot generation study (Jejurikar & Rohane, 2021) of protein-ligand interaction for human serum albumin (HAS) and four flavonoids present in himsagar mango, shows that hydrogen bonds, Pi-alkyl, Pi-Pi T shaped and Van der Waals interactions play significant roles in binding flavonoids to HAS (Figure 5).

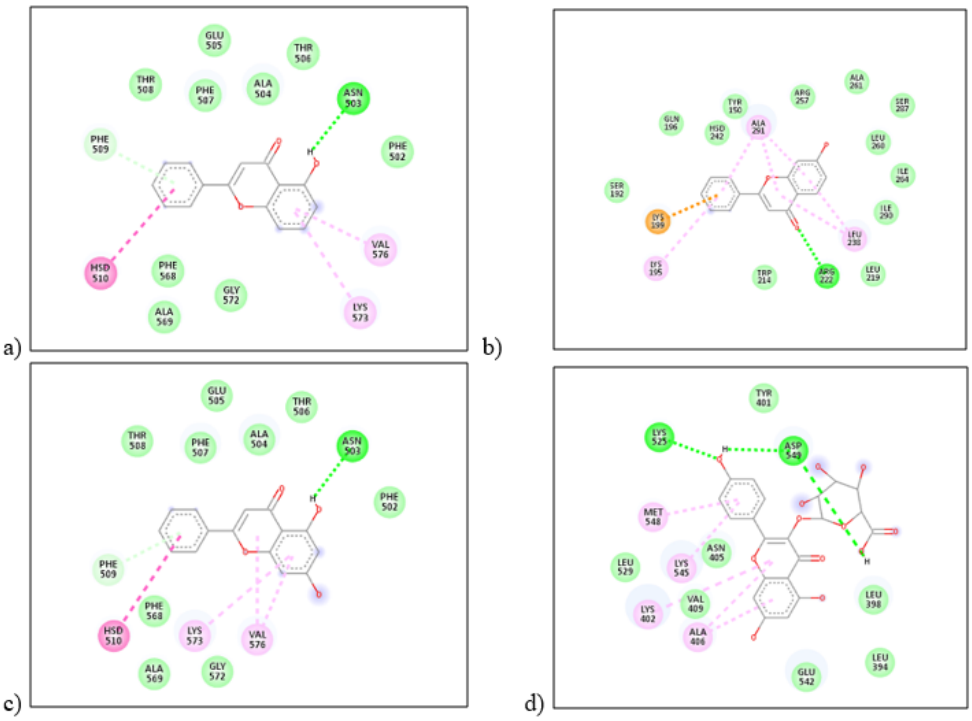


**Figure 5:** Generation of 2D Plot of Protein-Ligand Interaction for Human Serum Albumin and Flavonoids: a) Molecular Docking Structure of Albumin (Alpha Chain) with Primuletin; b) Molecular Docking Structure of Albumin (Alpha Chain) with 7-Hydroxy Flavones; c) Molecular Docking Structure of Albumin (Alpha Chain) with Chrysin; d)Molecular Docking Structure of Albumin (Alpha Chain) with Kaemferol-3-Glucuronide

The hydroxylation on 5 and 7 positions of A ring of flavone structure increases the affinities of ligands towards human serum albumin protein. Apart from the hydroxylation on 5 and 7 positions, the glycosylation on 3 position of C ring structure of flavone residue,

also causes affinity induction for the same protein. The molecular docking study reveals that, in addition to hydrogen bonds, Pi-alkyl, Pi-Pi T-shaped, and Van der Waals interactions (Figure 5) also play significant roles in the binding of flavonoids found in mango pulp to human serum albumin.

## Discussion

Drugs, fatty acids, vitamins and minerals, bilirubin, metabolites, and amino acids are among the endogenous and exogenous substances that human serum albumin (HAS) transports in blood (Varshney *et al*., 2010; Siddiqui *et al*., 2021; Leboffe *et al*., 2020). HSA's remarkable capacity to reversibly bind a variety of medications in plasma (Tayyab & Feroz, 2021) allows it to regulate their pharmacokinetics, osmotic pressures, and distribution patterns to distinct target tissues during plasma circulation (Zhivkova, 2015; Fanali *et al*., 2012). On the other hand, weak binding has been linked to poor drug absorption, delayed drug delivery rates, and delayed drug reach of action sites, while strong binding has been linked to an increase in release time and a subsequent decrease in the therapeutic values of drugs. The preferred model protein for researching the physiochemical and as well as biophysical behaviors of drug transports to target tissues is HSA, due to its moderate binding capacity (Kratz, 2008; Rahimizadeh, Yang & Lim, 2020).

A single polypeptide chain comprising 585 amino acid residues in three structurally related domains (I, II, and III) forms the basis of human serum albumin, which is divided into two subunits and joined by 17 disulfide bonds (Kragh-Hansen, 2016; Park *et al*., 2021). The polypeptide chain creates a structure that resembles a heart and has a thickness of 30 Å and approximate dimensions of 80×80×80 Å3. In the three structurally homologous domains (I, II, and III), approximately 67% of HSA is composed of α-helices, with no β-sheets (He & Carter, 1992; Belinskaia *et al*., 2020). There are ten helices in each domain; helices 1-6 make up sub-domains A, and helices 7–10 make up sub-domains B. The sub-domains IIA and IIIA contain two hydrophobic pockets where aromatic and heterocyclic ligands bind. The sub-domain interfaces, IIIA, IIIB, and IB are home to seven binding sites for fatty acids. Sudlow 1 and Sudlow 2 are the names of the two main binding sites found in subdomains II and III. For HSA, a number of additional binding sites have been found (Fig. 6) (Salem *et al*., 2019; Wang *et al*., 2022).

Using in-silico molecular docking, it was discovered that HAS has a moderate binding capacity when compared to compounds like primuletin (-6.90 Kcal/mol) and kaempherol-3-glucuronide (-8.87 Kcal/mole). Chrysin selectively exhibits exothermic binding on six binding sites, with binding energies ranging in −7.05 Kcal/mol. With a binding energy of −6.98 kcal/mol, 7 hydroxy flavone exothermally bound to a new large pocket on subdomain IIA (Sudlow 1).

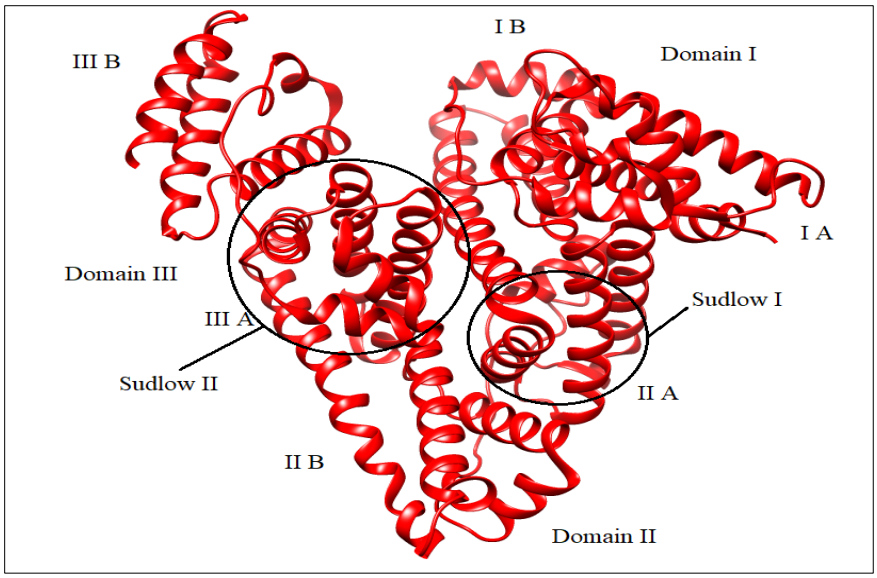


**Figure 6:** Domains, Subdomains and Binding Sites of Human Serum Albumin

## Conclusion

These findings supported the formation of stable HSA complexes involving kaempherol-3-glucuronide and 7 hydroxy flavones. They also advanced our knowledge of the binding properties of these compounds, including their affinities towards HSA, binding sites, mode of bindings, and non-covalent interactions involved in binding, as well as the structural alterations of HSA that occur during interactions. Strong binding between kaempherol-3-glucuronide and 7 hydroxy flavone has been linked to longer release times and a subsequent decline in the therapeutic benefits of medications, whereas weak binding of primuletin, has been linked to slower drug absorption, delayed delivery rates, and delayed action site reach. It has been demonstrated through molecular docking studies that HSA is capable of solubilizing and transporting chrysin compounds through blood with a moderate binding affinity to target tissues. This information is important for ascertaining the pharmacological characteristics of the four phytochemical compounds and their future development as anticancer therapeutic agents.

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