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Molecular Docking Studies on the Binding Interaction and Stability of Ovalbumin with 4-Dicyanomethylene-2,6-Dimethyl-4H-Pyran (DDPYRA) Dye in the Presence of Flavonoids

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KEYWORDS
Ovalbumin; 4-Dicyanomethylene-2,6-Dimethyl 4-H pyran; flavonoids; amino acid; molecular docking: hydrogen-bonding: hydrophobic interactions.

ABSTRACT:

Introduction: Molecular docking (MOD) techniques were employed in establishing the binding affinity and stability of Ovalbumin (OVA) with 4-Dicyanomethylene-2,6-Dimethyl-4-H-pyran (DDPYRA) dye in the presence of various flavonoids derived from *Psidium guajava*. OVA, a globular protein serves as the host, and DDPYRA acts as the guest.

Objectives: Flavonoids as competing guest molecule was docked simultaneously to establish the affinity of host-guest complex based on the stability and molecular interactions. The various dye-OVA conformers differ in energetics and molecular interactions, are compared with that of dye-OVA-flavonoids complex.

Methods: MOD is utilised as an effective tool and non-evasive technique for determining the stability of guest (dye)/competing guest (Flavonoids) with host (protein) is provided in depth.

Results: Docking studies reveal that the complexes of OVA-flavonoids and OVA-DDPYRA complexes are energetically less stable compared to that of OVA-DDPYRA-flavonoids complex. The binding stability is attributed to several conventional hydrogen-bonding (cHB) interactions existing between the amino acid (AA) residues of OVA with flavonoids. DDPYRA predominantly acts as hydrogen-bonding (HB) acceptor, and the protein as the donor. HB interactions predominate over hydrophobic interactions in the OVA-DDPYRA- flavonoids complex. Docking of flavonoids to the OVA-DDPYRA complex enhances the binding stability.

Conclusions: MOD studies further elucidate that dye is bound to several AA residues through cHB and non-conventional hydrogen-bonding (NcHB) interactions accompanied by hydrophobic interactions and weak van der Waals forces such that the introduction of flavonoids promotes more number of bimolecular interactions.

1. Introduction

Molecular docking (MOD) studies have gained importance in the field of study of host- guest interactions that acts as a bridge connecting chemists and biophysicist in the concept of molecular interactions, energetics and binding stability **[1-8].** However, research in the areas of diagnosis and treatment with respect to the concept of host-guest interactions comprising biomolecules (host) with fluorophores/drugs/metal ions/ligands (guest) is one of the most fascinating subject and significant domain of study through experimental and theoretical methods **[9-12].** The combination of electro analytical and photophysical studies in combination with MOD techniques aid in determining the nature of binding and molecular interactions existing between guest molecules (based on their solubility) with large globular proteins (Host) **[13-17].** Among the techniques, theoretical studies gained prominence particularly wherein both the guest and host molecules exhibit lesser solubility in water compared to hydrogen-bonding (HB) solutes. The most reliable data in the presence of several molecular interactions and the extent of binding stability could be ascertained with better reliability. Further, the results that are obtained provide us subtle information on the nature of the molecular interactions in the absence of water in a lesser time period with reliability. These characteristics presumably establishes the primary

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interaction governing the complex are either through HB, hydrophobic, electrostatic, or a weaker force of interaction. The primary binding forces in the development of the dye-protein complex establish through analytical techniques ably supported by MOD methods lead to conformational stability based on the above-mentioned interactions. Using MOD methodology, the energetically most feasible and favoured site of docking and orientation of guest/competing guest molecules within the different sub domains of the protein molecule has been determined and established such that it paves way for additional information at the molecular metabolism involving medicinal chemistry.

In addition to these parameters, the energetics provides an estimate of the level of stability generated by the complex formation of guest with host. Ovalbumin (OVA) is a globular protein, widely employed in the emerging fields of immunology to study immune responses, particularly in the allergic reactions and the development of the immune system**[18-21]**.OVA is categorized as a serpin family member and is less soluble in water for which docking studies with drugs and ligands provides an important information on the binding affinity. OVA based on its capacity to induce allergic reactions is considered to be significant in health aspects for mankind. OVA, a major egg white protein is highly regarded as a prevalent dietary protein regarding food chemistry. It has a large potential that serves as a carrier for unstable bioactive compound. Since OVA can be acquired in huge quantities from poultry, it is considered as a valuable protein in many areas of research, including general investigations of protein structure and characteristics. In the present investigation, the guest is DDPYRA dye which is generally referred as a derivative of dicyanomethylene (DCM) type **[22].** The well-known Intramolecular Charge Transfer (ICT) dye 4-Dicyanomethylene2,6-Dimethyl4-H pyran (DDPYRA) is an excellent example of a fine representation of a donor-acceptor molecule (figure 1). The methyl and the electron-accepting dicyano functional groups make up the electron-donating and electron-accepting nature of DDPYRA that has been imparted in the interaction studies involving non-fluorophoric solutes that promote electron transfer (ET) in solution **[23,24].** The presence of guest molecule with protein, competing host molecules with protein and competing host-guest interaction with protein are compared and explored through binding stability by using MOD studies **[25].** An in-depth exploration on the energetics involved in the formation of the OVA-DDPYRA-flavonoid complex found to be superior than that of either OVA-flavonoid or OVA-DDPYRA complex.

The structure of DDPYRA and the protein are provided in **figures 1 and 2** respectively**.**

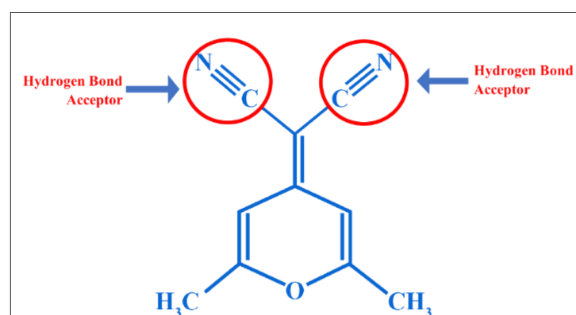

Figure 1: Structure of DDPYRA dye

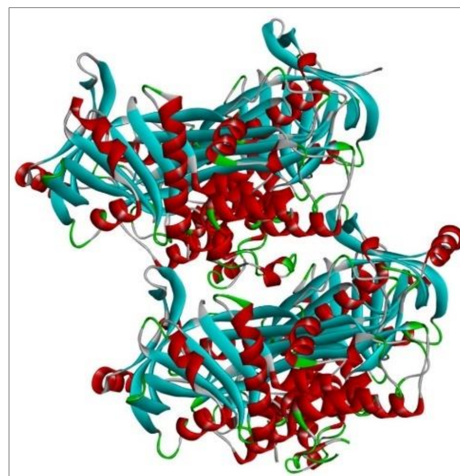

Figure2: PDB structure of OVA

(http://www.rcsb.org/pdb, PDB ID:1OVA)

Ovalbumin

OVA, the major protein in egg white, make up about 54% of its total protein content. It has attracted considerable interest in various fields due to its versatile functional properties. OVA is a moderately water-soluble glycoprotein, under specific conditions with a molecular weight of around 45 kDa. It is composed of 385 amino acids, providing a rich source of essential amino acids. Its stability, ease of purification, and well-defined structure has established it as a model protein in biochemical research **[26-28].** OVA is a well-known allergen and play a crucial role in allergy research **[29-31].** It aids in understanding the mechanisms of allergic reactions and in developing therapeutic interventions. Its multifunctionality highlights its significance across various fields. Current research continues to reveal new applications and deepen our understanding of this

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essential protein in the presence of several guest molecules.

2. Objectives

Importance of flavonoids

Psidium guajava (L.), a member of the Myrtaceae family, is a significant fruit in tropical regions **[32-35].** Research has highlighted the health benefits of guava leaves, which are attributed to various phytochemicals (Figure 3) including Phloretin (PHL), Naringenin (NAR), Apigenin (API), Luteolin (LUT), Quercetin (QUE), Isorhamnetin (ISO), Kaempferol (KAE), Myricetin (MYR), Morin (MOR), Gallic Acid (GAL), Catechin (CAT), Epicatechin (EPI), Gallo Catechin, Epigallocatechin Gallate, Leucocyanidin (LEU), and Delphinidin (DEL) **[36].** The phenolic compounds in guava leaves extract help to cure cancerous cells and prevent skin aging. They have the potential to be incorporated into the formulation of functional foods and pharmaceuticals. Guava plant as such including leaves, fruits and seeds has a high content of important antioxidants and has radio-protective ability.

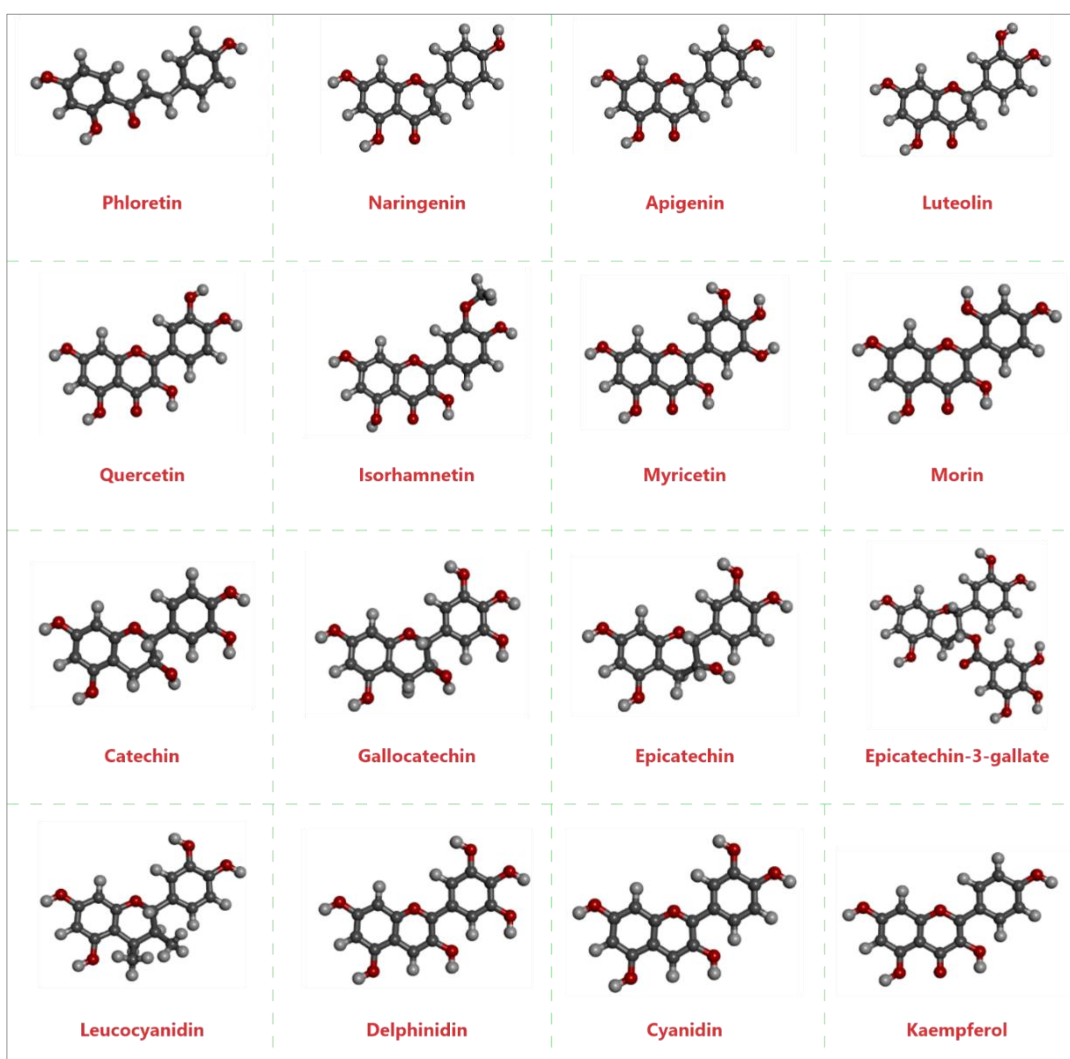

Figure 3: 3D structure of flavonoids employed in docking studies.

3. Methods

Molecular docking (MOD) studies

The Chemsketch tool was used to draw the structures of DDPYRA dye and flavonoids. The Mol format files for these structures were then converted to PDB format using Open Babel molecular converter software. The crystal structure of OVA was retrieved from protein databank (PDB) (http://www.rcsb.org/pdb, PDB ID: PDB 1OVA) **[37]** as shown in figure 2. The water molecules and complexes were removed during the binding interaction studies. OVA preparation was carried out using Autodock software version 4.2 as

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carried out in the studies involving of acridinedione dyes **[38-40].** The polar hydrogen and kollman charges were added and saved in pdbq format. Structures of OVA and dye were uploaded; centre node and torsional bonds were selected and saved in pdbqt format.The procedure was followed as reported **[41,42].** Finally, all the ten conformation was selected and saved in pdb format. OVA-DDPYRA complex formed were visualized using Biovia discovery studio visualizer and analyzed for HB, hydrophobic, van der Waals interactions as well as for any unfavorable interactions existing during the complex formation **[43].** Further docking with flavonoids, the formation of

OVA-flavonoids, OVA-DDPYRA and various OVA-DDPYRA-flavonoids complexes were carried out and the same procedure has been followed.The study examines flavonoids as the competing guest molecules alongside the fluorophore DDPYRA docked to ovalbumin. The flavonoids identified as competing ligands in the docking studies obey the Lipinski rule of Five **[44]** are illustrated in the figure 3 and the SMILES notation are provided in table 1. The research provides an in-depth analysis of the energetics and molecular interactions of DDPYRA with OVA, both in the absence and presence of the flavonoids

4. Results

The structures of dye and drug were determined using Lipinski rule of five. Certain flavonoids deviate from the rule based on the number of hydrogen-bonding acceptor sites, which can be considered. This methodology was used to calculate the energies and interactions with OVA in docking studies to find the stable conformers. The Molinspiration results of DDPYRA are reported elsewhere **[45]** and those of the flavonoids used in the present study are provided in Table 1 among which only five flavonoids exhibit a slight deviation based on the number of HB_D atoms.

Table 1: **SMILES notation of flavonoids**

Flavonoid	TPS A^2	Number of HB_D atoms	Number of HB_A atoms	Molecular weight	Heavy atom count	Rotatable Bond Count	Lipinski Rule
Phloretin	78	3	4	274	20	4	Obey
Naringenin	87	3	5	272	20	1	Obey
Apigenin	87	3	5	270	20	1	Obey
Luteolin	107	4	6	286	21	1	Obey
Kaempferol	107	4	6	286	21	1	Obey
Quercetin	127	5	7	302	22	1	Obey
Isorehmentin	116	4	7	316	23	2	Obey
Myricetin	116	**6**	8	318	23	2	Partially Deviate
Morin	127	5	7	302	22	1	Obey
Catechin	107	5	6	290	21	2	Obey
Gallo catechin	131	**6**	7	306	22	1	Partially Deviate
Epicatechin	110	5	6	290	21	1	Obey
Epicatechin-3-gallate	177	**7**	10	442	31	4	Partially Deviate
Leucocyanidin	131	**6**	7	302	22	1	Partially Deviate
Delphinidin	122	**6**	7	303	22	1	Partially Deviate
Cyanidin	102	5	5	287	21	1	Obey

Table 2: Energetics of all the conformers of DDPYRA dye with OVA.

Conformation	Binding Energy (BE) $kcalM^{-1}$	Ligand efficiency	Inhibitory constant, K_i (mm)	Intermolecular energy $kcalM^{-1}$	vdW + Hbond + desolv Energy $kcalM^{-1}$
DOVA01	-6.27	-0.48	25.29	-6.27	-6.26

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DOVA02	-6.26	-0.48	25.57	-6.26	-6.23
DOVA03	-6.16	-0.47	30.45	-6.16	-6.16
DOVA04	-6.04	-0.46	37.69	-6.04	-5.98
DOVA05	-6.04	-0.46	37.66	-6.04	-5.98
DOVA06	-6.04	-0.46	37.33	-6.04	-5.99
DOVA07	-6.00	-0.46	39.78	-6.00	-5.95
DOVA08	-5.40	-0.42	110.6	-5.40	-5.34
DOVA09	-5.39	-0.41	112.81	-5.39	-5.33
DOVA10	-5.32	-0.41	126.44	-5.32	-5.29

In DDPYRA, the two CN groups have nitrogen atoms that significantly contribute to molecular interactions through cHB. Besides HB with amino acid residues, DDPYRA also forms hydrophobic and weak van der Waals interactions. These interactions assist in the stabilization of the resulting conformers. With a molecular mass of 356 g/mol, (which is under 500 g/mol), the fluorophore is well-suited for docking studies. OVA is suitable for docking interactions with ligands because it primarily binds to hydrophobic molecules [**46**]. Lipinski rule is based on specific criteria and formulations for ligands (typically drugs) interacting with proteins or large macromolecules. According to this rule, the ligands should have a logP value of 5 or less, molecular weight of 500 g/mol or less. Further the rules state that ligand should not contain no more than 10 hydrogen bond acceptors (HB_A) and 5 hydrogen bond donors (HB_D). Based on these specifications, docking of DDPYRA, OVA and flavonoids were carried out by generating stable conformers. The energetics related to the formation of dye-protein complex and the molecular interactions arising from the complex formation are provided in table 2 and table 3 respectively. In the present study regarding the docking of OVA with dye and flavonoids, we have sub-divided the results and discussion part related to the energetics, bimolecular interactions and favourable binding sites and domains.

i) The first part of docking deals with the molecular interaction parameters and energetics of OVA-dye complex in the absence of flavonoids which is represents as host guest complex.

ii) The second part deals with the molecular interaction parameters and energetics of OVA with various flavonoids in the absence of dye wherein flavonoids are considered as the competing guest molecules.

iii). A comparison on the various parameters deals with the simultaneous docking of the various flavonoids to OVA-dye complex is discussed in depth which is categorized as role of competing guest molecules on the binding stability host-guest complex.

Further the number of active binding sites in which the dye molecule and along with flavonoids bound to the protein are determined. The binding of guest or competing guest molecules in helices/sheets/coils terms provide a depth of information regarding the binding sites.

Table 3. DDPYRA dye with OVA molecular interactions.

Conformer	HB D-A linkage of Amino acid (Atom···Ligand atom)	Hydrophobic interactions
OVADDPYRA1		Alkyl: Lys236: Pi-Sigma: Lys236:
OVADDPYRA2	Asn146(C…N)	Alkyl: Ile145, Leu156 Pi-Alkyl: Val347: Pi-anion:Glu122
OVADDPYRA3	Lys199(N…N) Val348(N…N)	Pi-cation: Lys199 Pi-donor: Ile224:

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		Pi-alkyl : Tyr222,Ile224 Alkyl:Val348
OVADDPYRA4	Asn146(C….N)	Pi-alkyl: Val347 Pi-anion:Glu122
OVADDPYRA5	Gly87	Pi-Sulphur: Cys133 Alkyl: Cys87, Cys133, Leu66, Leu127 Pi-alkyl: Tyr130
OVADDPYRA6	Gly87	Pi-Sulphur: Cys133 Alkyl: Cys87, Cys133, Leu66, Leu127 Pi-alkyl: Tyr130
OVADDPYRA7		Pi-sigma: Lys236
OVADDPYRA8	Asn146(C…N)	Pi-anion:Glu122 Alkyl: Ile145, Leu156 Pi-alkyl: Val347
OVADDPYRA9	Thr104(O…N) Ser116(C….O)	Pi-sigma: Leu114 Alkyl: Leu114, Arg139,Met63 Pi-alkyl: Arg139, Tyr138
OVADDPYRA10	Phe112(N…N) Asn107(N…N)	Alkyl:Pro106 Pi-alkyl: Pro106,Pro107

We have employed the MOD technique to explore the nature of bimolecular interaction existing between OVA with DDPYRA. In OVA-DDPYRA interaction, the dye is found to reside in a different active site of OVA, and the presence of flavonoids influences the energetics and binding sites of the dye-protein complex. Additionally, the MOD technique was used to estimate the BE of the dye with OVA for each conformer, revealing that the energetics of each conformer differs significantly in the presence of flavonoids. Similarly, the energetics are compared for the individual OVA-flavonoid complex and the OVA-DDPYRA-flavonoid complex. The binding sites of OVA with DDPYRA resulted in distinct conformers, which were obtained using Auto Dock 4.2 software. The structures of OVA with dye in presence of various flavonoid complexes were arranged based on their corresponding energetics ($kcalmol^{-1}$) with the formulation of several parameters leading to the stability of the complex. We optimized ten distinct conformations of OVA-DDPYRA, OVA- flavonoid, and OVA-DDPYRA- flavonoid. These structures were classified primarily based on their BE compared to other energy parameters, resulting in the identification of the most stable configuration for host-guest, host-competing guest, and host-guest-competing guest complexes.

Table 2 provides the complete energetics of dye protein complex. The electrostatic energies vary in the range of -0.03 ± 0.03. In all, the conformers exhibit torsional energy values are zero. Value of zero implies

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minimal strain, allowing the ligand to adopt various conformations. Further total internal unbound energy is also zero which indicates stable, unstrained unbound complex exist between dye and protein. The ten different conformers and their structures were arranged based on the binding energy (kcal mol^{-1}) and corresponding energetics parameter that comprises the formulation of several intermolecular forces leading to the stability of dye-protein binding complex. Ten different conformations of DDPYRA-OVA were generated and the optimized structures were labeled from DOVA01 TO DOVA10. The energetics related to the formation of various conformers of DOVA complex and the molecular interaction parameters are provided in Table 3 respectively.

From Table 2 it is postulated that both DOVA1 and DOVA2 are energetically most stable complexes. The conformers DOVA4 to DOVA7 possess similar BE. Among the ten conformers DOVA8, DOVA9 and DOVA10 are energetically less stable than most stable conformer. Further it has established that inhibitory constant value above 100 is set to be unstable. Herein the energetically least stable conformers that exhibits value above 100 in the host- guest complex are considered to be less stable. Table 3 present the molecular interactions formation of DDPYRA-OVA conformers, along with molecular interaction parameters (including HB interactions, hydrophobic interactions, and weak interactions such as pi-pi, pi-alkyl, and electrostatic interactions). Similarly, Tables 4 and 5 provide information on the energetics of OVA with various flavonoid conformers and OVA-DDPYRA-flavonoid conformers, respectively. In general, the most stable conformer resulting from the docking of dye or drug with a protein is associated with a minimal BE and a lower inhibitory constant value (ki). Beyond these factors, complex formation is favoured when intermolecular energy and ligand efficiency (dye or drug) exhibit fewer negative values. Additionally, the role of torsional energy in complex formation, along with total internal unbound energy parameters, contributes to the stability and ease of complex formation. Comparing these parameters reveals an intriguing contrast between dye-bound and drug-bound protein molecules.

OVA-DDPYRA dye conformer analysis

The interactions that govern the stability of host-guest-competing host complex were determined by docking studies. Based on the software (Biovia Discovery studio visualizer) provided through MOD technique, the binding constants of DDPYRA dye-OVA conformers along with their binding sites in OVA were determined. In general, the most stable conformer formed due to docking of the guest molecule (drug/ fluorophore/ligands) with the host molecule (protein) is correlated to minimum binding energy that is accompanied with a lesser inhibitory constant value (ki). Apart from these parameters, formation of a complex is most favoured when the intermolecular energy and the ligand efficiency values are less negative. In the case of DDPYRA dye with OVA, the most energetically stable binding complex corresponds to DOVA01 conformer and the least stable complexes are those which have a very high inhibitory constant value above 100 (Table 2). Based on the above parameters, the conformers DOVA08 to DOVA10 possesses a very high binding energy compared to that of DOVA01- DOVA07 conformers. Moreover, the intermolecular energy and solvation energy parameters make unfavourable complex formation such that the relative stability of all other complexes is relatively lesser compared to conformers DOVA01 and DOVA02. Further, on closer examination of these structures also provided whether the HB or hydrophobic influences govern the stability of the complexes.

Conformer DOVA01: Being energetically the stable conformer, the dye does not form any type of conventional or non-conventional hydrogen-bonding interaction and this conformer is stabilized through hydrophobic interactions only. Interestingly, Lys 236 is the only amino acid that is involved in pi-alkyl bonding interactions which is categorized as hydrophobic interaction.

Conformer DOVA02: This conformer has very similar BE to that of DOVA01 and all the energetic parameters of DOVA02 are similar to that of DOVA01, except in terms of the amino acids involved in interaction. Asn146 forms a cHB with nitrogen atom of CN moiety of DDPYRA dye. Glu, Ile, Leu and Val are the amino acids that are involved in hydrophobic interaction with dye. Interestingly, the BE being similar for both the conformers, yet they differ completely in the nature of binding interaction such that the amino acids that are involved in molecular interactions are entirely different. Likewise, the amino acids that are involved in HB in conformer 3 (DOVA3), are through Lys199 and Val348 and the contribution through hydrophobic interactions are through five different amino acids namely Ala, Glu, Ile, Lys and Val. Compared to conformers DOVA1 and DOVA2, the contribution from amino acids that are involved in complex formation is found to be higher in the case of DOVA3. Interestingly, the fourth and the eighth most stable conformers based on BE (DOVA4 and DOVA8) complex has a similar molecular interaction pattern as that of DOVA2 (Table 3) which clearly reveals that the binding site and domains of these three conformers (DOVA2, DOVA4 and DOVA8) are similar.

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On a closer analysis, the conformers DOVA5 and DOVA6 are stabilized predominantly by hydrophobic interactions that consists of pi-alkyl, alkyl-alkyl and pi-sulphur binding. These two conformers are represented by only one HB interaction through glycine amino acid. All other conformers exist predominantly through hydrophobic interactions and among the conformers generated, conformers DOVA1 and DOVA7 does not possess any HB, whereas all other conformers possess as well as hydrophobic interactions. A detailed illustration regarding the number of molecular interactions, the nature of amino acids (hydrophilic or hydrophobic) involved in complex formation and the position of the amino acid sequence in OVA is provided in table 3. The energetics of DOVA1 and DOVA2 even though similar to each other in energetic, the stabilization through the molecular interactions are entirely different. Likewise, the energetics of DOVA4 = DOVA5 = DOVA6 = DOVA7 are quite similar to each other with very marginal differences in their inhibitory constant.

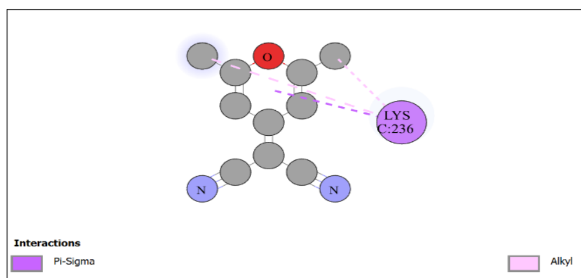

Figure 4a

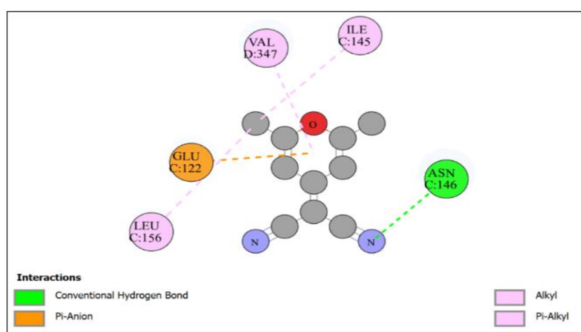

Figure 4b

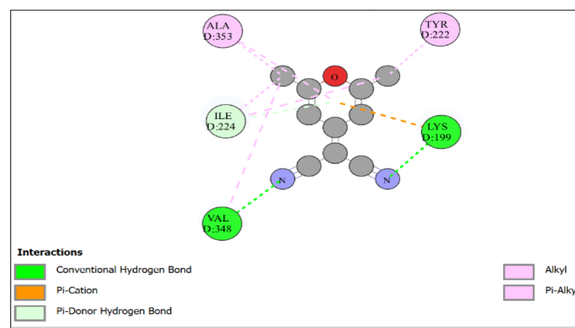

Figure 4c

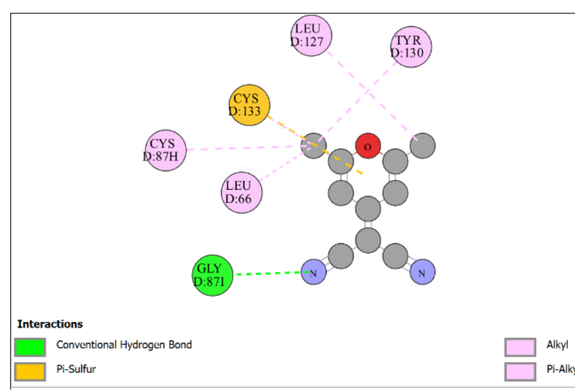

Figure 4d

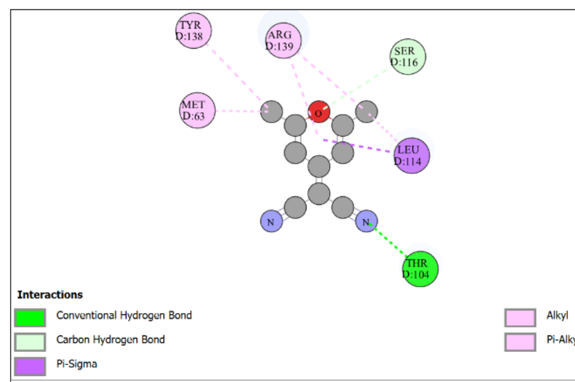

Figure 4e

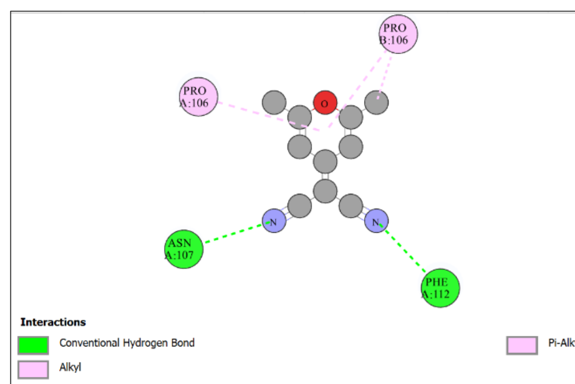

Figure 4f

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Figure 4 (a-f): 2D structure of unique conformations of dye OVA structures. **4a:** DOVA01 and DOVA07, **4b:** DOVA02, DOVA04 and DOVA08, **4c:** DOVA03,**4d:** DOVA05 and DOVA06, **4e:** DOVA09, **4f:** DOVA10

However, the formation of a complex is most favoured and stable when the intermolecular energy and the ligand efficiency values are less negative in addition to BE. Additionally, the significance of torsional energy and total internal unbound energy parameters also account for the stability of the complex. However in our case both the values are found to be zero. A comparison on the competing guest molecule with host versus dye with protein were carried out.

OVA- FLA conformer analysis

The energetics related to the docking of various flavonoids with OVA resulting in various conformers is provided in table 4. The free energy associated with the complex formation between flavonoids and protein is classified according to thermodynamic parameters. In addition to BE, various other parameters contribute to the stabilization of the complex. Based on BE values, Kaempherol docked with OVA exhibit most stable binding energy of -7.49 kcal/mol, indicating the highest stability among the listed conformations. However Isorehmetin docked with protein exhibit least negative binding energy of -3.74 kcal/mol, indicating the lowest stability among the various flavonoids docked with OVA. The most stable competing guest with host is with Kaempherol > Delphinin > Catechin > Gallo catechin represented as KAEOVA, DELOVA, CATOVA and GATOVA respectively. Among the flavonoids five flavonoids exhibit with larger binding energy dye docked with protein. Whereas 11 other flavonoids exhibit much lesser binding energy than the dye molecule. Our focus of study revolves around the role of flavonoids on dye protein complex due to their highly negative binding energies and strong intermolecular interactions. The least stable conformations are with Isorehmentin (ISOOVA) and Epigallocatechin (EPIGAOVA). The stability is due to their more positive binding energies and lower contributions from key stabilizing energies.

OVA-DDPYRA-Flavonoids conformer analysis

Simultaneous docking of the flavonoids with dye OVA complex resulted larger enhancement of binding stability. Among the 16 flavonoids considered in the study, all the flavonoids strengthen the complex formation existing between dye and protein except Morin and Myricetin. From table 5 it is evident that competing guest molecules which are the flavonoids not only stabilize the dye protein complex rather their binding energies also increases to more negative value when docked with dye protein complex. A cumulative and associative binding of the flavonoids with dye protein complex is visualized clearly in the study. Detailed study on the stabilization of dye protein complex on the introduction of flavonoids is provided in the table 4 and figure 10.

The protein contains several amino acid residues that form the chain sequence. The amino acids are classified into active and non-active binding sites based on the position of the amino acid[]. There are 5 active sites in OVA represented as site 1, site 2, site 3, site 4 and site 5. Several guest molecules dock any of these sites independently or in combined form. A detailed amino acid residue of each binding sites is provided in the literature [**47-49**] that comprises several polar and non-polar amino acid. Reports on OVA with an ICT based dye as the guest molecule reveals that the energetically stable 10 conformer are docked in different binding sites of OVA, whereas with that of a flavonoid (competing guest) does not prefer to reside in all the binding sites. Further, the various binding sites comprises helices to turns, sheets and coils in the protein structure such that the energetically stable conformer of the guest molecule with OVA provides a detailed information of the preferred binding sites. Phloretin, apigenin, luteolin and Gallo catechin does not prefer to reside in any of the active binding site of dye-protein complex. In the present study, as many 16 flavonoid when docked to dye - OVA complex, fewer flavonoids namely phloretin, apigenin, luteolin and Gallo catechin does not prefer to reside nor dock in any of the five active binding sites in OVA. The most favoured active binding sites in OVA is site 5 for several flavonoids, whereas Isorehmentin, Cyanidin and Epicatechin-3-gallate prefers to residue in two different sites namely site 5 and site 1. Similarly, Quercetin and Delphinidin bind in two active sites namely site 2 and site 3. Apart from the above-mentioned flavonoids, though most of the flavonoid differ only in the number of HB donor and acceptor moieties primarily, yet the variation in the active clearly reveals that the mechanism and mode of binding differs significantly in the presence of DDPYRA dye. Energetically, almost all the flavonoid stabilizes the complex existing between dye and protein except for the case of Myricetin and Morin. Both Myricetin and Morin destabilize the complex, while others are involved in stabilization of the dye-protein complex. The stabilization of the dye-protein complex by flavonoids is primarily through HB, though pi- Sigma and pi -alkyl interaction are also exhibited.

A closer analysis of the molecular interactions existing between flavonoids in the presence of dye-OVA complex reveals several interesting characteristics.

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Phloretin and Apigenin involve only one amino acid (Lys) involved in binding with protein, wherein both the flavonoids are stabilized by hydrophobic interaction only. Apart from these two, all other flavonoids exhibits five and above molecular interactions. Both HB and hydrophobic interaction co exist in determining the stability of the complex. The binary stability of certain flavonoids like Luteolin, Quercetin, Myricetin and Gallo catechin are governed by HB interaction only in comparison to that of hydrophobic interaction. However, in other than the mentioned flavonoids, all other binding with DDPYRA - OVA complex are governed equally by HB and hydrophobic interactions. They are Naringenin, Isorehmentin, Catechin, Epicatechin3-gallate, Leucocyanin and Cyanidin. The above information is clearly represented in Table3.

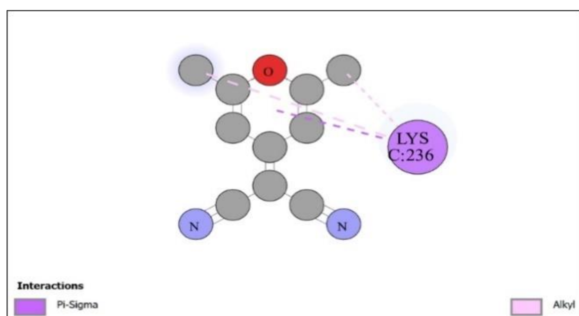

Figure 5a

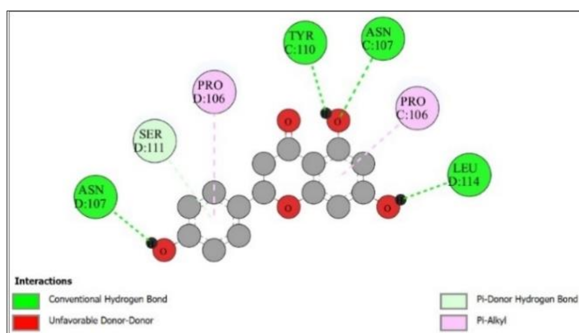

Figure 5b

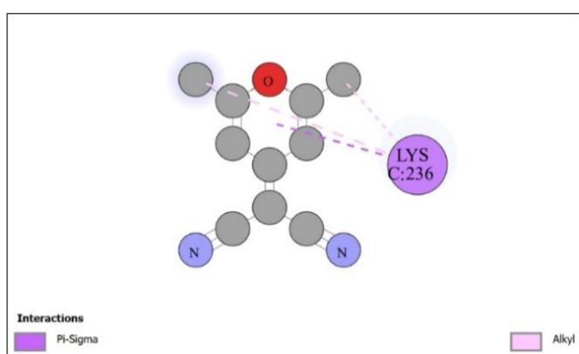

Figure 5c

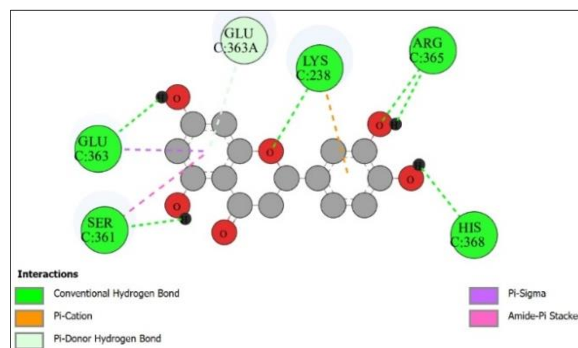

Figure 5d

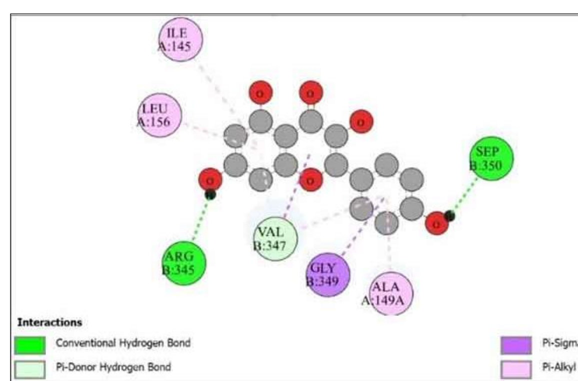

Figure 5e

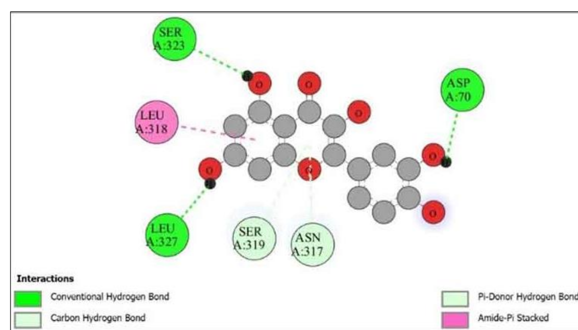

Figure 5f

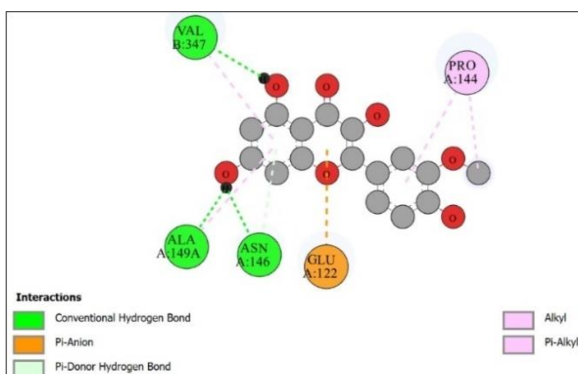

Figure 5g

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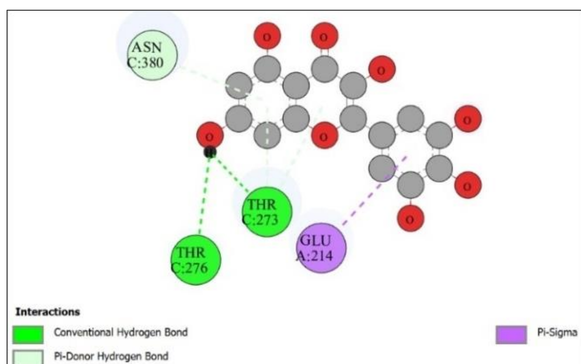

Figure 5h

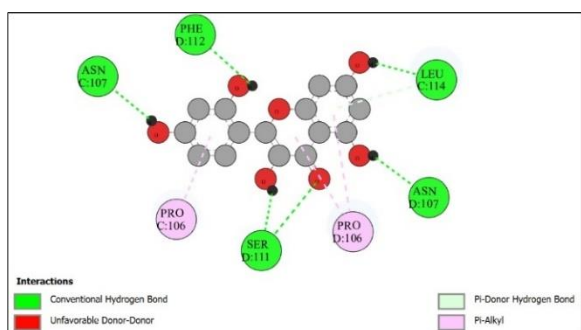

Figure 5i

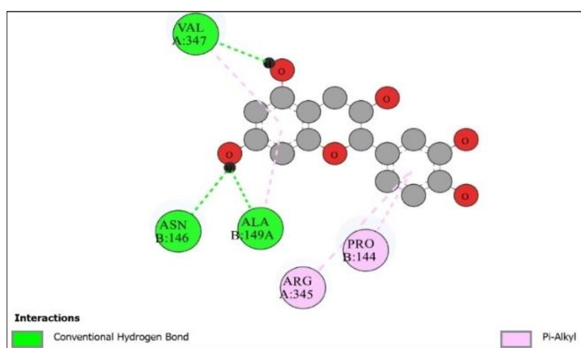

Figure 5j

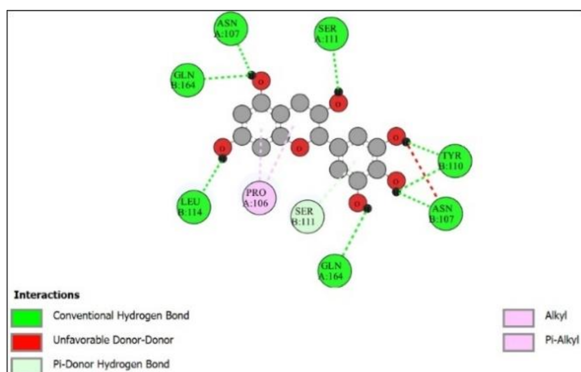

Figure 5k

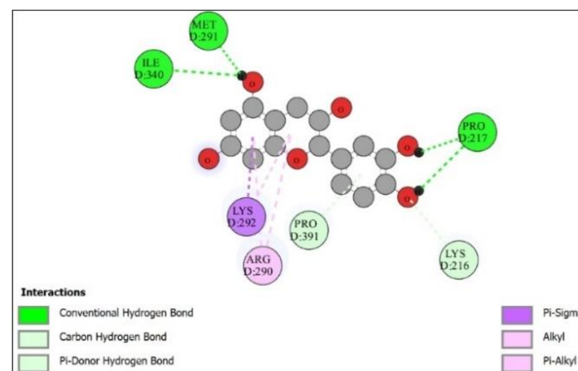

Figure 5l

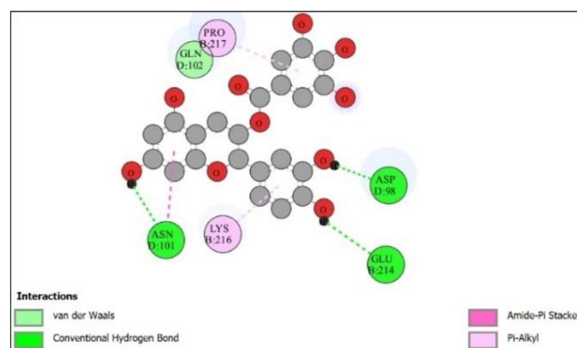

Figure 5m

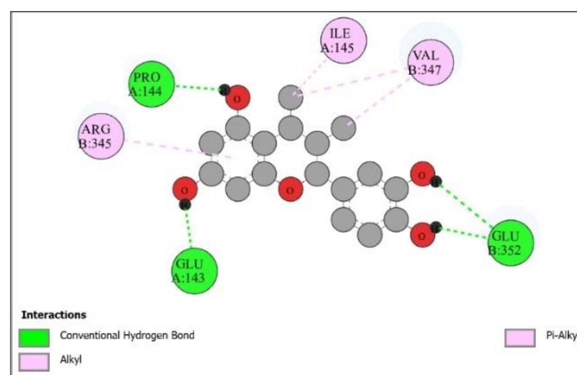

Figure 5n

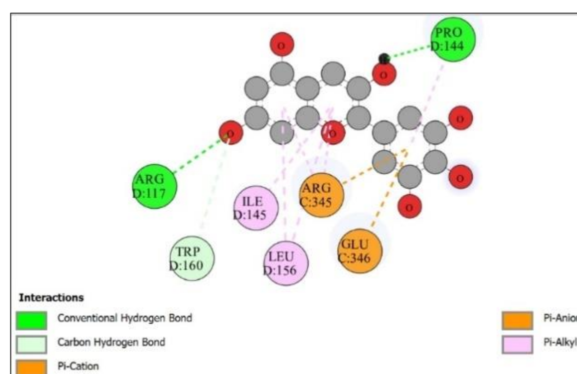

Figure 5o

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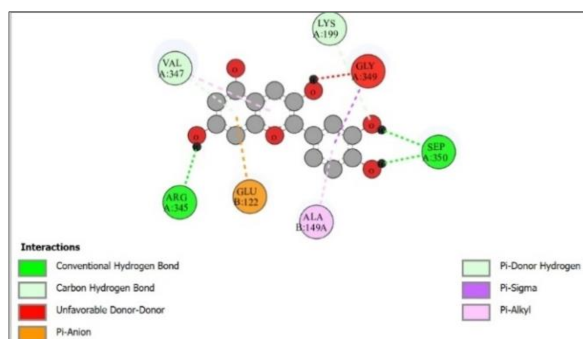

Figure 5p

Figure 5(a-o): 2D structure of stable structure of dye-OVA complex with all the flavonoids **5a:** DOVAPHL, **5b:** DOVANAR, **5c:** DOVAAPI, **5d:** DOVALUT, **5e:** DOVAKAE, **5f:** DOVAQUE, **5g:** DOVAISO, **5h:** DOVAMYR, **5i:** DOVAMOR, **5j:** DOVACAT, **5k:** DOVAGAL, **5l:** DOVAEPI, **5m:** DOVAEPIGA, **5n:** DOVALEU, **5o:** DOVADEL, **5p:** DOVACYAN

As from the analysis, the docking score of dye when docked to protein in the absence of flavonoids is -6.27 Kcal/mol and simultaneous docking of all the flavonoids to the host-guest complex predominantly increases the binding affinity of the Dye-OVA complex except in the case of myricetin. Among the flavonoids DEL, GAL, NAR and API are the competing guest molecules that results in higher stability compared to others.

Table 4: Representation of variation in BE and bimolecular interactions of Host-Guest versus Host-Guest-Competing Guest interactions.

Flavanoid	Binding Energy of Dye-OVA complex with flavonoids	Binding Energy of Dye-OVA complex	Difference in B.E in Kcalmol^{-1}	Inference	Molecular interactions
Phloretin	-6.72	-6.27	-0.45	stabilizes	Pi -Sigma interactions
Naringenin	-7.61	-6.27	-1.34	stabilizes	HB, Pi -donor, Pi-alkyl
Apigenin	-7.58	-6.27	-1.31	stabilizes	Pi -sigma, Pi-alkyl
Luteolin	-7.42	-6.27	-1.15	stabilizes	HB,Pi -cation
Kaempferol	-7.7	-6.27	-1.43	stabilizes	HB, Pi-sigma, Pi-alkyl
Quercetin	-7.15	-6.27	-0.88	stabilizes	HB, Pi -sigma interactions
Isorehmentin	-6.68	-6.27	-0.41	stabilizes	HB,Pi -anion, Pi-alkyl
Myricetin	-5.94	-6.27	0.33	destabilizes	HB,Pi -sigma
Morin	-6.22	-6.27	0.05	destabilizes	HB,Pi-alkyl
Catechin	-7.24	-6.27	-0.97	stabilizes	HB,Pi-alkyl
Gallo catechin	-7.65	-6.27	-1.38	stabilizes	HB,Pi-alkyl
Epicatechin	-7.4	-6.27	-1.13	stabilizes	HB, Pi-Sigma, Pi-alkyl
Epicatechin-3-gallate	-7.4	-6.27	-1.13	stabilizes	HB,Amide-Pi, Pi-alkyl
Leucocyanidin	-6.38	-6.27	-0.11	stabilizes	HB,Pi-alkyl
Delphinidin	-7.78	-6.27	-1.51	stabilizes	HB, Pi- Sigma, Pi-alkyl, Pi-cation, Pi-anion
Cyanidin	-6.54	-6.27	-0.27	stabilizes	HB, Pi-alkyl, Pi-cation, Pi-anion

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It is obvious that the flavonoids exhibit varying effects on the stability of the dye-OVA complex. Certain flavonoids stabilize the complex through multiple interactions that differ in nature of the amino acid and the functional groups that are involved in interactions. The stabilisation/destabilisation based on the functional groups in flavonoids probably does not govern the nature of study since all the plant extracts considered in the study contains three acceptors moieties and more donor moieties. All the flavonoids dock through HB in certain cases and all involve some sort of hydrophobic interaction as provided in (supporting data table 2 and 3). Epicatechin-3-gallate stands out markedly due to its unique interactions within the dye-OVA complex. Unlike other flavonoids, it engages in both HB and amide-Pi interactions, contributing to its enhanced stability. Myricetin and Morin exhibit positive BE differences, indicating certain extent of destabilization. Naringenin stabilizes the complex through HB, pi-donor interactions and through pi-alkyl interactions. Both,

Apigenin and Kaempferol stabilizes the complex via pi-sigma interactions and pi-alkyl interactions. Luteolin stabilizes the complex through HB and pi-cation interactions. Quercetin stabilizes the complex through HB and pi-sigma interactions. Isorhamnetin stabilizes the complex via HB, pi-anion interactions, and pi-alkyl interactions. Catechin and Gallo catechin stabilizes the complex via HB and pi-alkyl interactions. Among these

flavonoids, Delphinidin exhibits the more interaction with the OVA-DDPYRA complex. Delphinidin exhibits remarkable stability. Its BE with the complex is -7.78 kcal/mol. In the absence of delphinidin, the BE of the complex is more positive. The difference in BE is -1.51 kcal/mol establishes that the presence of competing solute role on the complex varies based on the individual flavonoid. Diverse interactions such as cHB, NcHB, pi-sigma interactions, pi-alkyl interactions, pi-cation interactions, and pi-anion interactions contribute to the stability of the complex.

The Table 4 presents a variety of interaction types involving different residues and functional groups. Pi-sigma and Pi-alkyl interactions are prevalent, indicating significant involvement of aromatic and aliphatic residues in stacking and hydrophobic contexts. Pi-sigma bonds are found in Kaempferol, luteolin, Epicatechin, Epicatechin-3-gallate and Cyanidin. Alkyl hydrophobic interaction is found in Phloretin and Gallo catechin. Pi-donor interactions often involve hydrophobic environments, with residues like Leu114 and Asn146 participating in these interactions. Pi-donor hydrophobic interactions are evident in Morin. Apigenin , Isorehmentin and Epicatechin-3-gallate interactions suggest that lone pair electrons (often from oxygen or nitrogen in amide or anion groups) are involved in hydrophobic interactions with nearby nonpolar groups. Pi-anion interactions are noted in specific residues such as Glu346 and Glu122,

demonstrating interactions between negatively charged residues and pi systems. Pi-anion hydrophobic interaction is evident in Delphinidin and Epicatechin. Apigenin and Leucocyanidin contains pi-cation hydrophobic interactions. Unfavorable interaction found in Morin, Gallo catechin, Epicatechin-3-gallate and Cyanidin. Weak Van der Waals forces of attraction and unfavorable interactions falls under the category of other interactions. Phloretin and Delphinidin do not involve any HB. Amide pi-stacking interactions which typically involve the interaction between the amide group and aromatic rings or other pi systems, are found in Epicatechin-3-gallate. This indicates the presence of an amide group interacting in a pi-stacking or hydrophobic context with another pi system.

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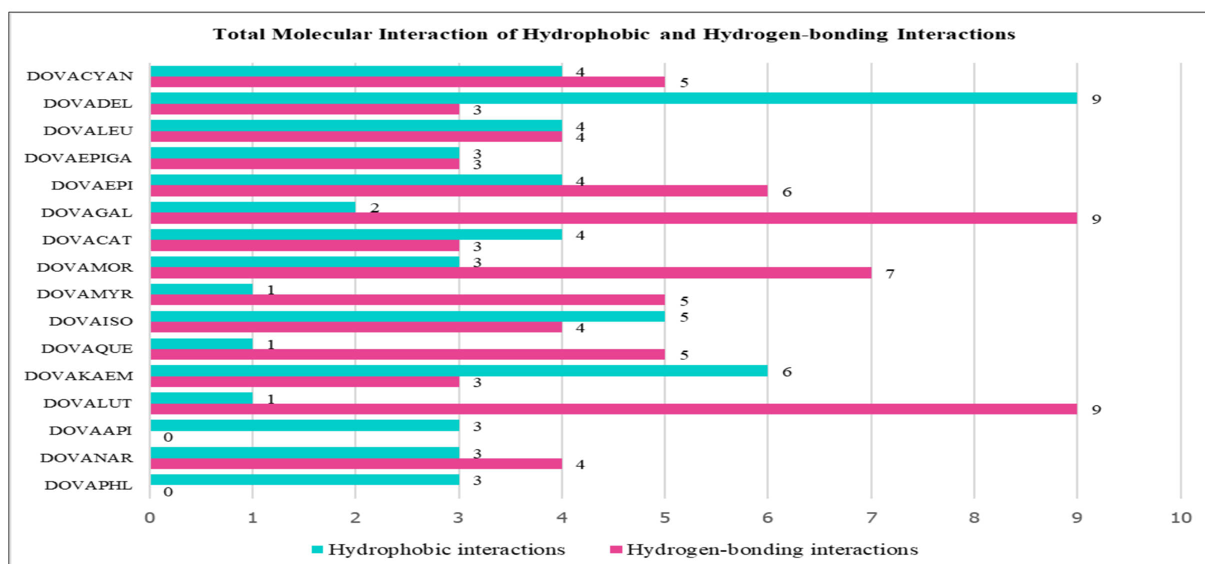

Figure 6: Total Molecular Interaction of Hydrophobic and Hydrogen-bonding Interactions

On analyzing the data of molecular interactions resulted from (supporting data table 3), figure 6, Gallo catechin exhibits the maximum number of interactions. Gallo catechin engages in 12 molecular interactions with various amino acids, making it one of the most interactive flavonoids in the complex. The presence of 9 HB interactions contributes significantly to stability such that HB interactions predominate over hydrophobic interactions. It forms 2 hydrophobic interactions, further enhancing complex stability. Its diverse interactions set it apart from other flavonoids. Further, the favourable binding BE and specific interactions make it a key role in the complex formation. Myricetin exhibits the least number of interactions. It engages in 6 molecular interactions, making it one of the least interactive flavonoids in the complex with 5 HB and 1 hydrophobic interaction. All the complexes exhibit more number of HB than hydrophobic interactions. HBs are crucial for stabilizing molecular complexes. They involve attractive interactions between hydrogen atoms and electronegative atoms (such as oxygen or nitrogen). Hydrophobic interactions occur between nonpolar molecules. These interactions are less specific and weaker than HBs. Phloretin, Apigenin, Kaempferol, Isorehmentin, Catechin and Delphinidin have more hydrophobic interactions than HBs. Morin, Gallo catechin, Epicatechin-3-gallate, Cyanidin have interactions other than HBs and hydrophobic.

Table 5: Polar and non-polar Amino acids involved in OVA-DDPYRA-Flavanoid conformers

Flavanoid	Polar Amino acids involved in interactions	Non-Polar Amino acids involved in interactions
Phloretin	Lys	NIL
Naringenin	Asn, Ser, Tyr	Leu, Pro
Apigenin	Lys	NIL
Luteolin	Arg, Glu, His, Lys, Ser	NIL
Kaempferol	NIL	Ala, Gly, Ile, Leu, Val
Quercetin	Asn, Asp, Ser,	Leu

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Isorehmentin	Asn, Glu	Ala, Pro, Val,
Myricetin	Asn, Glu Thr	NIL
Morin	Asn, Ser	Leu, Phe, Pro
Catechin	Arg, Asn,	Ala, Pro, Val
Gallo catechin	Asn, Gln, Ser, Tyr	Leu, Pro
Epicatechin	Arg, Lys	Ile, Met, Pro
Epicatechin-3-gallate	Asp, Glu, Asn, Lys	Pro
Leucocyanidin	Arg, Glu	Ile, Pro, Val
Delphinidin	Arg, Glu, Lys,	Ala, Val
Cyanidin	Arg, Glu, Lys	Ala, Val, Gly

All amino acids are definitely involved when a flavonoid is docked with dye-protein complex. Asparagine (Asn) amino acid of the protein involves with more than 8 flavonoids and the most frequently involved polar amino acids are Asparagine (Asn), Lysine (Lys), Glutamic acid (Glu), Serine (Ser) and Arginine (Arg). On the other hand, the non-polar amino acids are Proline (Pro), Leucine (Leu) and Valine (Val) which widely involved rather than other amino acids. On the other side, the least involved polar amino acids are Histidine (His),Threonine (Thr), Phenylalanine (Phe) and Glutamine (Gln) which are involved only once. Similarly, least frequently involved non polar amino acids are Phenylalanine (Phe) and Methionine (Met). Phloretin, Apigenin, Luteolin and Myricetin does not involve any non-polar amino acid in bimolecular interaction. Similarly, Kaempferol alone does not involve polar amino acid interaction. Luteolin docked with dye-protein consists of maximum number of polar amino acids Kaempferol consists of maximum number of non-polar amino acids. Based on the nature of amino acid that are involved in HB or hydrophilic interactions, the contribution from the polar amino acids are predominately involved in molecular interactions except in the case of kaempferol (Figure 7).

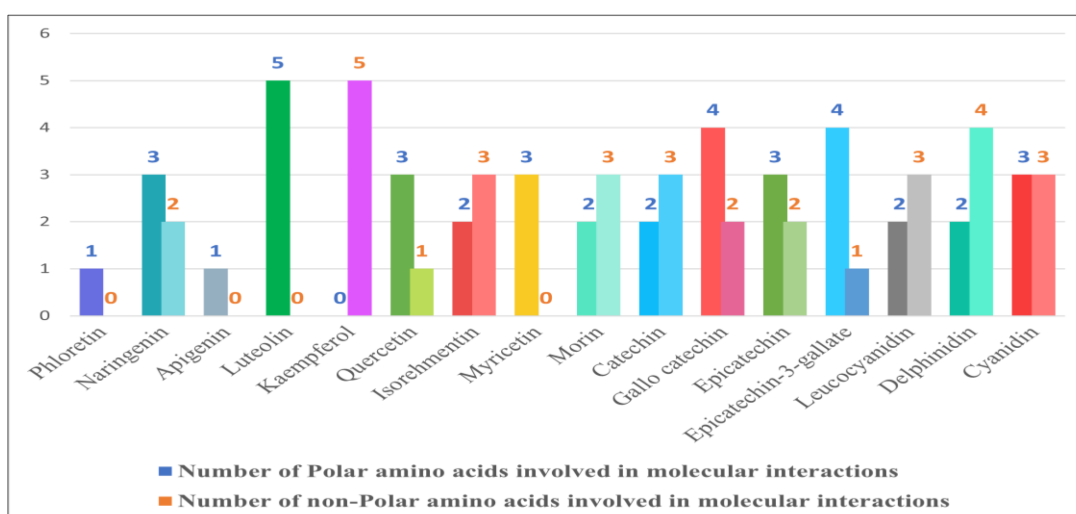

Figure 7: Polar and Non-polar Amino acids involved in OVA-DDPYRA-FLA conformers

In all OVA-DDPYRA-FLA, the binding site 5 is most preferred binding site whereas all other binding sites are equally preferred. Binding site 5 is only preferred site for DOVAKAE, DOVAMYR and DOVACAT. Two preferred binding sites, site 2 and site 3 are involved in DOVAQUE and DOVADEL. Binding site 4 are involved only in DOVANAR, DOVAMOR

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and DOVALEU. Only one binding sites preferred flavonoids are Naringenin, Luteolin, Kaempferol, Myricetin, Morin, Catechin, Epicatechin and Leucocyanidin (Figure 8) and the overall representation based on the binding site and domain of the sequence of the amino acid bound, an illustrative representation is provided in figure 9.

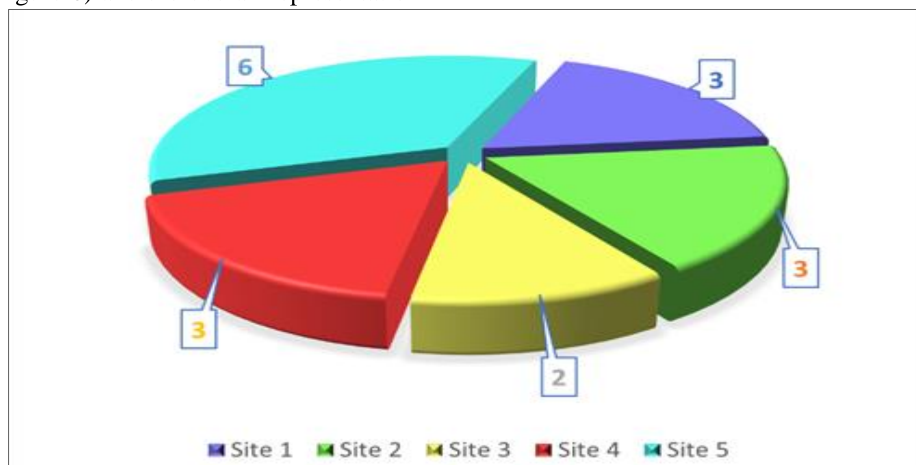

Figure 8: Pie chart representation on preferred binding site of OVA-DDPYRA-Flavonoids

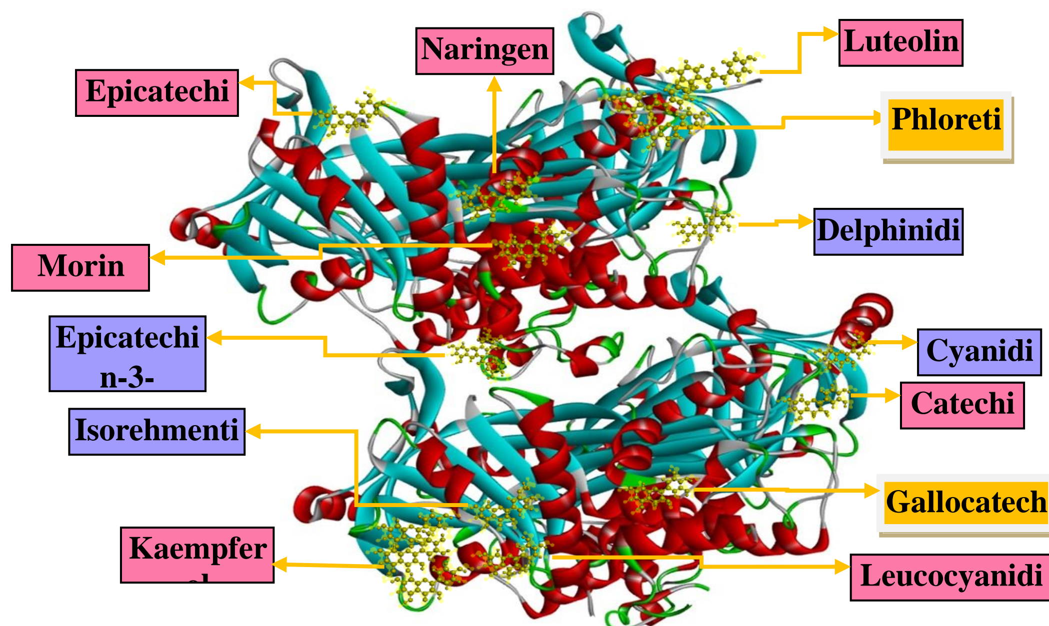

Figure 9: Overall binding site representation of dye-OVA conformer with flavonoids

By employing molecular docking techniques, it is evident that the simultaneous docking of flavonoids to OVA-dye complex is stable rather than OVA-dye nor OVA-flavonoid complexes (Figure 10). Based on the binding site specificity on OVA, docking of various flavonoids is not easily optimized even though all the extracts obtained from guava plant has similar structural properties regarding HB_A and HB_D, whereas the dye possesses a more favourable pattern of docking with OVA. The combination of protein with dye and flavonoids results in a more stable interaction compared to host-guest interactions. Within OVA, there exist various sub domains wherein both the flavonoids and dye reside without any unfavorable interactions. The

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co-existence of multiple binding sites with different energies (stable conformer) explains the intricate interactions between OVA with DDPYRA, and flavonoids. The stability is predominantly attributed to HB interactions wherein along the coexistence of hydrophobic interactions also contribute towards the stability of the conformers and binding domains.

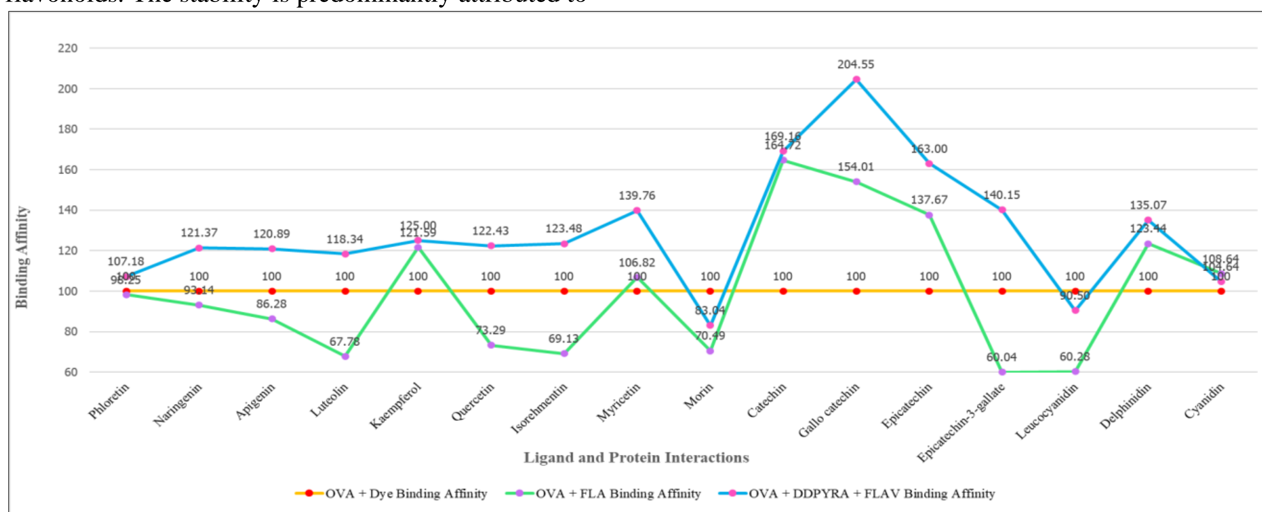

Figure 10: Stabilization of OVA-DDPYRA-Flavanoid complex

5. Discussion

Based on the overall docking studies, the outcomes are summarized for in depth understanding on the energetics (Figure 10) and molecular interactions.

i) OVA-DDYRA complex energy is -6.27 kcal/mol, whereas that of OVA-flavanoid complex energy ranges from -3.17 to -7.49 kcal/mol based on the structure.

ii) The complex stability is rationalised based on the binding affinity of flavonoid with OVA complex and it ranges from 60 % to 164 % compared to that of dye-OVA complex. However, flavonoids are introduced, the stability of the OVA-dye complex increases considerably.

iii) Protein-dye-flavanoid energy values range from -5.94 to -7.78 kcal/mol and its binding affinity ranges from 83% to 204 %. This complex exhibits enhanced stability among all.

iv) The total number of interactions between the Protein-dye conformers and OVA-FLA conformer is comparatively less than OVA-DDPYRA-FLA. In the OVA-DDPYRA conformer, HB is less and it is even absent in OVADDPYRAYRA1 and OVADDPYRAYRA7 conformer. but hydrophobic interactions dominate.

v) Molecular Interactions: In OVA-FLA have more HB and it dominates the hydrophobic interaction. But when OVA-DDPYRA interacts with FLA, a greater number of hydrophobic interactions are observed.

vi) Interestingly, an unfavourable bond occurs when OVA-DDPYRA docks with Myricetin, Catechin, Epicatechin-3-gallate and Cyanidin. Over all, OVA-DDPYRA and OVA-FLA complexes are stabilised by HB whereas OVA-DDPYRA-FLA stability is depending on hydrophobic interactions.

vii) Participation of Amino Acids: In OVA-DDPYRA lesser number of amino acids are involved whereas both OVA- FLA and OVA-DDPYRA-FLA complexes shows various number of amino acids are involved.

viii) Phloretin, Apigenin, Luteolin and Myricetin does not involve in interaction with non-polar amino acids. Kaempferol relies only on non-polar interactions and not on the Polar amino acid. Non- polar amino acid Methionine (Met) is not involved in any of the molecular interactions except with Epicatechin

ix) In the OVA-DDPYRA dye interactions, the amino acid Lysine (LYS) appears to be most involved. It participates in alkyl interactions and pi-sigma interactions.

x) whereas in the interactions involving OVA-DDPYRA conformers, the amino acid Asparagine (ASN) appears to be most involved. It participates in HB

xi) interactions with other amino acids. polar amino acids are more preferred than non polar amino acids as shown in the fig 7

xii) Preferred binding site: In OVA-DDPYRA-FLA, binding site 5 is most preferred. All other sites are

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equally preferred when OVA-DDPYRA docked with various FLA as shown in the fig 9

6. Conclusions

Molecular docking has been employed as a simple, efficient and reliable tool in ascertaining the forces that govern the binding stability of various naturally occurring flavanoids possessing five or six membered cyclic ring structures when docked with OVA-DDPYRA dye system. The stability of the host-guest or host-competing guest interactions is influenced by hydrogen bonding accompanied by several hydrophobic interactions. The variation in the energetics and stability of the host-guest systems are ascertained accurately which the most significant factor through in silico studies is. Theoretical model employed in ligand systems that exhibits lesser solubility in water comprising a bimolecule provides an accurate and precise approach toward the binding sites and sub domains of the protein molecule. Further, the nature of the amino acids that are involved in binding play a key role on the stability is imparted in the present study. The presence of similar structures exhibited by the flavonoids varying in the substituent in the phenyl and cyclohexyl ring prefers to reside in different active binding sites of Ovalbumin are established by MOD studies.

Declaration of competing interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data providing Supporting information tables

1. Table ST1: Energetics of all the TEN conformers of OVA with various flavonoids.
2. Table ST2: Energetics of all the TEN conformers of OVA-Dye complex when simultaneously docked with various flavonoids
3. Table ST3 : Number of bimolecular interactions of stable conformer of OVA-Dye complex when simultaneously docked with various flavonoids
4. Table ST4: Preferred binding sites of stable conformer of OVA-Dye complex when simultaneously docked with various flavonoids

Table ST1: Energetics of all the TEN conformers of OVA with various flavonoids.

Conformation	Binding Energy (B.E)	Ligand efficiency	Inhibitory constant, K_i (mm)	Intermolecular energy	vdW+ H	Electrostatic energy	Torsional energy	Total Internal

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					bond + desolv Energy			Unbound energy
PHLOVA	-6.16	-0.32	30.7	-8.24	-8.12	-0.12	2.09	-1.11
NAROVA	-5.84	-0.29	52.7	-7.03	-6.81	-0.22	1.19	-0.93
APIOVA	-5.41	-0.27	108.9	-6.6	-6.25	-0.35	1.19	-0.91
LUTOVA	-4.25	-0.2	772	-5.74	-5.55	-0.19	1.49	-1.74
KAEOVA	-7.49	-0.33	3.26	-8.98	-8.78	-0.19	1.49	-2.06
QUEOVA	-4.28	-0.19	732	-6.07	-5.69	-0.38	1.79	-2.07
ISOOVA	-3.74	-0.16	1.83	-5.53	-5.4	-0.13	1.79	-1.76
MYROVA	-4.54	-0.19	26.9	-6.07	-5.69	-0.34	1.79	-2.05
MOROVA	-5.28	0.24	135	7.07	6.92	-0.15	1.79	-1.51
CATOVA	-7.05	-0.34	6.82	-8.84	-8.69	-0.15	1.79	-0.73
GALOVA	-7	-0.32	7.37	-9.09	-8.97	-0.13	2.09	-1.46
EPIOVA	-6.25	-0.3	26.2	-8.04	-7.87	-0.18	1.79	-1.08
EPIGAOVA	-3.17	-0.1	4.72	-6.45	-6.08	-0.37	3.28	-3.67
LEUOVA	-4.25	-0.2	3.78	-5.74	-5.24	-0.19	1.49	-1.74
DELOVA	-7.11	-0.32	6.12	-9.2	-8.98	-0.22	2.09	-1.11
CYAOVA	-6.79	-0.32	10.52	-8.58	-8.33	-0.25	1.79	-1.06

Table ST2: Energetics of all the TEN conformers of OVA-Dye complex when simultaneously docked with various flavonoids

Conformation	Binding Energy (B.E)	Ligand efficiency	Inhibitory constant, K_i (μm)	Intermolecular energy	vdW+ H bond + desolv Energy	Electrostatic energy	Torsional energy	Total Internal Unbound energy
DOVA	-6.27	-0.48	25.29	-6.27	-6.26	-0.01	0	0
DOVAPHL	-6.72	-0.35	11.88	-8.81	-8.76	-0.05	2.09	-0.91
DOVANAR	-7.61	-0.38	2.62	-8.81	-8.8	-0.01	1.19	-0.93
DOVAAPI	-7.58	-0.38	2.8	-8.77	-8.78	0.01	1.19	-0.92
DOVALUT	-7.42	-0.35	3.64	-8.91	-8.77	-0.14	1.49	-1.72
DOVAKAEM	-7.7	-0.37	2.02	-9.26	-9.18	-0.08	1.49	-1.32
DOVAQUE	-7.15	-0.33	5.74	-8.94	-8.83	-0.11	1.79	-1.75
DOVAISO	-6.68	-0.29	12.64	-8.47	-8.45	-0.03	1.79	-1.78

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DOVAMYR	-5.94	-0.26	44	-8.03	-7.51	-0.52	2.09	2.43
DOVAMOR	-6.22	-0.28	27.49	-8.01	-7.98	-0.03	1.79	-1.83
DOVACAT	-7.24	-0.34	4.93	-9.03	-8.92	-0.11	1.79	-1.09
DOVAGAL	-7.65	-0.35	2.4	-9.74	-9.63	-0.11	2.09	-1.56
DOVAEPI	-7.4	-0.35	3.76	-9.19	-9.08	-0.11	1.79	-1.15
DOVAEPIGA	-7.4	-0.35	3.76	-9.19	-9.08	-0.11	1.79	-1.15
DOVALEU	-6.38	-0.29	20.9	-7.88	-7.36	-0.52	1.45	-1.12
DOVADEL	-7.78	-0.35	2	-9.86	-9.74	-0.12	2.09	-1.6
DOVACYAN	-6.54	-0.31	16.19	-8.33	-8.12	-0.21	1.79	-1.13

Table ST3 : Number of bimolecular interactions of stable conformer of OVA-Dye complex when simultaneously docked with various flavonoids

Conformer	HB amino acid	Bond distance	Hydrophobic interaction	Bond distance	Other interaction
DOVAPHL			alkyl: LYS236	4.46	
			LYS236	4.28	
			LYS236(π -sigma)	3.6	
DOVANAR	ASN107	2.36	SER111(π -donor)	3.71	
	TYR110	1.96	PRO106(π-alkyl)	5.31	
	ASN107	2.85	PRO106(π-alkyl)	5.31	
	LEU114	2.06			
DOVAAPI			LYS236(π-sigma)	3.6	
			LYS236(π-alkyl)	4.46	
			LYS236(π-alkyl)	4.28	
DOVALUT	SER361	2.29	LYS238(π-cation)	4.07	
	SER361	4.23			
	GLU363	3.6			
	GLU363	2.63			
	GLU363(π-donor)	4.04			
	LYS238	2.71			
	ARG365	2.9			
	ARG365	2.2			
	HIS368	2.42			
DOVAKAEM	ANG345	2.12	GLY349(π-sigma)	3.87	
	SEP360	2.12	VAL347(π-sigma)	3.71	
	VAL347(π-donor)	4.17	ALA149(π-alkyl)	3.63	
			ILE145(π-alkyl)	5.34	

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			LEU156(π-alkyl)	5.2	
			VAL347(π-alkyl)	5.48	
DOVAQUE	ASP70	2.06	LEU318	3.99	
	SER323	2.66			
	LEU327	1.82			
	ASN317	3.86			
	SER319	3.63			
DOVAISO	ASN146	2.58	PRO144(π-alkyl)	5.14	
	ALA149	2.04	ALA149(π-alkyl)	5.15	
	VAL347	2.61	VAL347(π-alkyl)	5.02	
	ASN146(sigma-donor)	4.2	PRO144(π-alkyl)	3.94	
			GLU122(π-anion)	4.67	
DOVAMYR	THR273	2.33	GLU214(π-sigma)	3.99	
	THR273	3.05			
	THR273(pi-donor)	3.83			
	THR273(pi-donor)	3.94			
	ASN380(pi-donor)	3.72			
DOVAMOR	ASN107	2.54	PRO106(π-alkyl)	4.68	ASN107
	PHE112	2.68	PRO106(π-alkyl)	4.78	
	LEU111	2.34	PRO106(π-alkyl)	4.69	
	ASN107	2.24			
	SER111	2.26			
	SER111	2.81			
	LEU114(pi-donor)	4.18			
DOVACAT	ASN146	2.4	PRO144(π-alkyl)	4.96	
	ALA149	2.15	ARG345	5.35	
	VAL347	2.55	ALA149(π-alkyl)	4.63	
			VAL347(π-alkyl)	5.35	
DOVAGAL	ASN107	2.04	PRO106(π-alkyl)	4.7	ASN107
	GLN164	2.49	PRO106(alkyl)	5.21	
	SER111	2.27			
	LEU114	2.11			
	GLN164	2.93			
	ASN107	2.56			
	TYR110	2.33			
	TYR110	2.06			
	SER111(π-donor)	3.55			
DOVAEPI	PRO217	2.07	LYS292(π -sigma)	3.87	
	PRO217	2.67	LYS292(alkyl)	4.28	
	MET291	2.14	ARG290(π-alkyl)	4.66	
	ILE340	3.04	ARG290(π-alkyl)	5.45	

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	LYS216	3.1			
	PRO391	4.16			
DOVAEPIGA	ASP98	1.97	ASN101(Amide-π)	4.81	GLN102
	GLU214	2.93	LYS216(π-alkyl)	4.29	
	ASN101	2.33	PRO217(π-alkyl)	4.27	
DOVALEU	GLU352	2.75	ARG345(π-alkyl)	5.25	
	GLU352	2.14	ILE145(alkyl)	5.19	
	PRO144	2.1	VAL347(π-alkyl)	5.38	
	GLU143	2.17	VAL347(π-alkyl)	5.2	
DOVADEL	ARG117	3.06	ARG345(π-cation)	4.87	
	PRO144	2.32	GLU346(π-anion)	3.63	
	TRP160	3.69	ARG345(π-alkyl)	4.5	
			ARG345(π-alkyl)	5.31	
			PRO144(π-alkyl)	4.88	
			ARG345(π-alkyl)	4.5	
			LEU156(π-alkyl)	5.48	
			LEU156(π-alkyl)	5.27	
			ILE145(π-alkyl)	4.89	
DOVACYAN	ARG345	2.18	GLY349(π-sigma)	3.43	GLY349
	SEP350	2.23	ALA149(π-alkyl)	3.78	
	SEP350	2.4	VAL347(π-alkyl)	5.29	
	LYS199	3.19	GLU122(π-anion)	4.69	
	VAL347(π-donor)	4			

Table ST4: Preferred binding sites of stable conformer of OVA-Dye complex when simultaneously docked with various flavonoids

Flavanoid	Site 1	Site 2	Site 3	Site 4	Site 5
Phloretin	**X**	**X**	**X**	**X**	**X**
Naringenin	**X**	**X**	**X**	**Favoured**	**X**
Apigenin	**X**	**X**	**X**	**X**	**X**
Luteolin	**X**	**X**	**Favoured**	**X**	**X**
Kaempferol	**X**	**X**	**X**	**X**	**Favoured**
Quercetin	**X**	**Favoured**	**Favoured**	**X**	**X**
Isorehmentin	**Favoured**	**X**	**X**	**X**	**Favoured**
Myricetin	**X**	**X**	**X**	**X**	**Favoured**
Morin	**X**	**X**	**X**	**Favoured**	**X**
Catechin	**X**	**X**	**X**	**X**	**Favoured**

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Gallo catechin	**X**	**X**	**X**	**X**	**X**
Epicatechin	**X**	**Favoured**	**X**	**X**	**X**
Epicatechin-3-gallate	**Favoured**	**X**	**X**	**X**	**Favoured**
Leucocyanidin	**X**	**X**	**X**	**Favoured**	**X**
Delphinidin	**X**	**Favoured**	**Favoured**	**X**	**X**
Cyanidin	**Favoured**	**X**	**X**	**X**	**Favoured**