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Molecular Docking Studies on Binding Sites, Interactions and Stability of Globular Protein, Ovalbumin (OVA) with 4-Dicyanomethylene-2-methyl-6-(4-dimethylaminostyryl)-4H-pyran (DCDAP) Dye in presence of various Flavonoids of *Psidium guajava*

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KEYWORDS

Ovalbumin, Flavonoids, DCDAP, Molecular docking, hydrogen-bonding, Binding stability

ABSTRACT:

Introduction: Molecular docking (Mol.Doc) techniques were employed to ascertain the binding sites, interactions and stability of a globular protein with an Intramolecular Charge Transfer dye in the presence of flavonoids extracted from guava fruit leaves. Ovalbumin (OVA) as the host, 4-dicyanomethylene-2-methyl-6-(4-dimethylaminostyryl)-4H-pyran (DCDAP) dye as the guest and the flavonoids as competing guest molecules are employed in the study.

Objectives: OVA as the host, DCDAP dye as the guest and the flavonoids as competing guest molecules are employed in the study to establish the bimolecular forces governing the stability of the host-guest complex in the presence of a competing guest molecule.

Methods: Mol.Doc studies were carried out using Autodock software version 4.2 as performed in the studies regarding dye-protein-drug system. In docking studies, Lamarckian genetic algorithm is applied. As the result of several conformers generated, the best 10 stable clusters were selected in the descending order of binding energy (B.E) conformation ranking. The 10 conformations were selected and further saved in pdb format. OVA-DCDAP complex was, further docked with a set of 16 flavonoids based on subject to Lipinski rule of Five.

Results: The host-guest conformers were categorized based on the docking score correlated to binding energy and the stability of the complex are governed by the bimolecular interactions. Studies reveal that protein-dye complex is relatively less stable than protein-dye-flavonoid complex. The stability is attributed to several conventional hydrogen-bonding interactions existing between dye-flavonoid and the amino acid residues of OVA, act as the hydrogen-bonding donor while dye acts as hydrogen-bonding acceptor. Molecular docking of flavonoid binding to protein-dye complex results in an enhanced stability of the complex. Further, there exists no direct binding of dye with flavonoid when docked simultaneously. Moreover, flavonoid and dye reside far apart from each other in distinct sites of OVA.

Conclusions: The stability upon complex formation is correlated to the docking of the guest molecule in the binding domains of OVA. The presence of multiple hydrophobic interactions such as pi-pi, pi-alkyl, pi-cation or anion, pi-sigma and pi-amide along with conventional hydrogen-bonding and weak van der Waals in OVA-dye -flavonoid complex does not destabilize, instead promote the binding stability.

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1. Introduction

4-dicyanomethylene-2-methyl-6-(4-dimethylaminostyryl)-4H-pyran (DCDAP) moiety, which consists of D-π-A system exhibits an excellent Intermolecular Charge transfer (ICT) property (**Figure1**). The luminescence mechanism of DCDAP family of probes is based on this ICT phenomenon and has been reported predominantly in non-aqueous solvents rather in water and polar solvents.[1-3] DCDAP dye consist of N, N-dimethylaniline group that acts as electron donor and dicyanomethylene group acts as electron acceptor, these two groups are covalently attached by a π – conjugated moiety of 4H- Pyran ring in the form of electron donor–electron acceptor (D-π-A) architecture. DCDAP fluorophore possess a single structed fluorescence band in non-polar aprotic solvents and it is not soluble in water. DCDAP is found to be entirely different from that of 4-dicyanomethylene-2, 6-dimethyl-4H-pyran (DDP) dye which has better solubility in water such that it acts as an excellent probe molecule to explore the photophysical properties in the presence of non-fluorophore solutes like derivatives of urea, amides, guanidine hydrochloride.[3-6]

The electronic excitation of DCDAP family of dyes results in the formation of locally excited (LE) state immediately after photoexcitation. Hence, the fluorescence emission of DCDAP in non-polar solvents occurs from LE state, formed through π- π* transitions and has electronic configuration similar to that of ground state. Hence, due to all these characteristics of DCDAP molecule the studies were primarily established on the basis of photo-isomerization and optical sensing applications only rather towards host-guest interactions.[7-9] Excited DCDAP molecules emitted from ICT state in polar solvents are characterized by a planar molecule confirmation, immediately formed after the photoexcitation under the influence of electric polarization of the surrounded solvent molecules. Short wavelength fluorescence is originated from ICT state and exhibits a gradual shift in the fluorescence band position from non-polar to polar solvents.[10] The excited DCDAP dye in the ICT state produces dual fluorescence with a twisted conformation due to internal rotation of the donor moiety along with ICT to an appropriate acceptor orbital.[14] Due to enhanced solubility of DDP dye, photophysical, electrochemical and theoretical studies with globular proteins were carried out which provides an excellent source of information regarding the nature of the binding and the forces that govern the stability of the host-guest complexes.[15] The present study with ovalbumin as the host in the presence of several flavonoids which acts as the competing guest molecules. The lesser soluble properties of DCDAP dye over DDP dye in water prompted us to carry out extensive docking studies on the factors influencing the complex affinity of dye-protein-flavonoid system. The structure of DCDAP and the protein is provided **(Figure1&2)** and that of flavonoids in supporting information **(SF 1).**

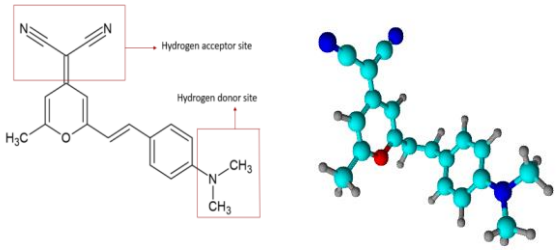

Figure 1: Structure of DCDAP dye

Ovalbumin (OVA), a widely studied globular protein contains 385 amino acid residues characteristic of one di-sulphide bond and four free sulfhydryl groups. OVA is the main protein found in egg white of molecular weight of 44.5 KDa has foaming and gelling properties. OVA is a member of the serine protease inhibitors (serpin) super family and is well established for it various biological properties that includes anti-cancer, anti-hypertensive, anti-microbial, anti-oxidant, immune system modulation properties. Further, the purified OVA contains different sub classes – A1, A2, A3 that contains two, one and zero phosphate groups which are of importance in metabolism for energy production. Ovalbumin is the first protein isolated in pure form and when denatured by heat, was found to have very high anti-mutagenic activity. Two and six ACE-inhibitory peptides, present in ovalbumin, exhibit antihypertensive activity. Among these peptides, two novel peptides with potent ACE-inhibitory activity have been found to reduce the vascular resistance and hence helpful in preventing and treating hypertension. An antihypertensive and Vaso relaxing octapeptide of ovalbumin, known as ovokinin, stimulates the release of prostacyclin that inhibits platelet activation.[11]

Peptides of ovalbumin, which were produced by digestion and were active against different strains of bacteria as well as fungi. Water-repelling properties and charge of these peptides are important for bactericidal activity. Significantly, it exhibits antimicrobial activity by restricting lipid oxidation. Hydrolysates of OVA, holds application in sport medicine as its consumption allows amino acid to be absorbed by the body more

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rapidly than intact proteins that maximizes nutrient delivery to muscle tissues. Thus, exhibit immune stimulatory property. Antioxidant activity can be enhanced by increasing lipid affinity. OVA induce the release of tumour necrosis factor (TNF), cell that protect the body by ingesting harmful foreign particles like bacteria or dead cells and thus possesses the immune-modulating activity of macrophages, a type of white blood cell of the innate immune system.[12]

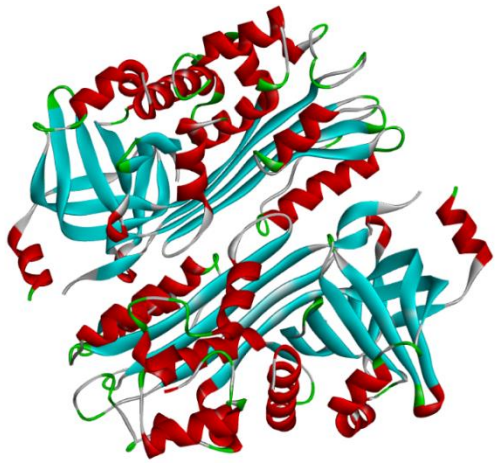

Figure 2: PDB Structure of Ovalbumin (PDB 1OVA)

Psidium guajava L. (guava plant) belongs to the Myrtaceae family is predominantly grown in various countries in both tropical as well in subtropical regions. Guava plant as a whole possesses several medicinal properties and is well known for treating various disorders such as skin problems, jaundice, cerebral ailments, diabetes, cancer, etc. The leaves of the guava plant in particular have been extensively studied for their health benefits which are attributed to their plethora of phytochemicals, such as quercetin, avicularin, apigenin, guaijaverin, kaempferol, hyperin, myricetin, gallic acid, catechin, epicatechin, chlorogenic acid, epigallocatechin gallate, and caffeic acid. Extracts from guava leaves (GLs) have been studied for their biological activities, including anticancer, antidiabetic, antioxidant, antidiarrheal, antimicrobial, lipid-lowering, and hepatoprotection activities. It is rich in phenolics, triterpenoids, tannins, vitamins, essential oils and sesquiterpene alcohols. [13,16]

2. Objectives

Studies of guava flavonoids on the basis of effects of air pollution, anti-cancer activities and anti-hyperglycemic activities have been explored and nearly 373 research articles on guava resulted of which 12 were related to cancer related treatment methodologies. Ethnobotanical survey of plants (Togo) used for the management of Type 2 diabetes (T2D) and hypertension exhibited that *Psidium guajava L*. plant imparts a significant role. Among the extracts, flavonoids appear to be the predominant compounds isolated from the guava plant and almost all classes of flavonoids exist to be isolated from or detected in the leaves and fruits. The flavonoids quercetin, kaempferol, guaijaverin, avicularin, myricetin and apigenin have been isolated from the leaves as part of a study for α-glucosidase and α-amylase inhibition studies. The present study involves flavonoids as the competing guest molecule along with the fluorophore (DCDAP) as the potential guest along with the host molecule (Ovalbumin). The absence and presence of flavonoids are discussed in depth.[17]

3. Methods

Chemsketch tool was employed to draw the structures of DCDAP dye and flavonoids. The MOL format files of these structures were converted to PBD format using open babel molecular converter software. OVA structure was retrieved from the protein data bank **(PDB 1OVA)** and the water molecules are eliminated from its structure for the binding interaction studies as carried out in docking studies regarding proteins**.** The Simplified Molecular Input Line Entry System (SMILES) format was generated using Chemsketch and their properties were calculated. Molecular docking (Mol.Doc) studies were carried out using Autodock software version 4.2 as performed in the studies regarding dye-protein-drug system. The polar hydrogen and Kollman charges were added and saved in the pdbq format. The structure of DCDAP as ligand were uploaded and torsional bonds were selected and saved in the pdbqt format. In grid preparation, the structure of flavonoids was saved in the pdbqt format, and later grid spacing was set between 0.750 Å to 0.850 Å with the grid box size of 126 Å × 126 Å × 126 Å, such that whole of OVA structure was accounted for. In docking studies, Lamarckian genetic algorithm is applied. As the result of several conformers generated, the best 10 stable clusters were selected in the descending order of BE conformation ranking. The 10 conformations were selected and further saved in pdb format. OVA-DCDAP complex was, further docked with a set of 16 flavonoids based on subject to Lipinski rule of Five.

The resulting conformers of OVA-DCDAP-flavonoid complexes were arranged based on their energetics as carried out for dye-protein complex. On the basis of energetics, best conformation of OVA- DCDAP -

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flavonoid complexes generated were taken for consideration, which is the first conformer. Minimum binding energy (B.E) obtained from docking of OVAD-DCDAP complex with flavonoid is the most stable conformer in consideration.[18]

4. Results

Binding of DCDAP dye with OVA

The energetics related to the docking of DCDAP dye with OVA resulting in various conformers is provided in **Table 1.** The energetics of all the interactions are represented in $kCalM^{-1}$. The torsional energy and the total internal unbound energy for all the conformers are found to be 0.89 and -0.45 $kcalM^{-1}$ respectively. The free energy obtained due to complex formation between dye and protein is categorized based on the thermodynamic parameters of which the free energy is referred as the B.E (Binding Energy) in docking studies. Apart from B.E, there are several other parameters involved in stabilization of the complex. The energetics includes several contributions like electrostatic, intermolecular and torsional energies. Moreover, molecular interactions such as weak van der Waals's forces of interaction, desolvation energy and hydrogen bonding (HB) also contribute for the stabilization of the dye-protein complex. The ten different conformers generated for DCDAP with OVA are assigned as OVA-DCDAP 1-10 respectively. Based on B.E values, the conformers are arranged in the decreasing order of stability accounting for the inhibitory constant values, intermolecular energy and parameters involving ligand efficiency. The larger the negative value of B.E indicates the ease of formation and stability of the complex which are correlated with thermodynamic parameters.

In the presence of water molecules, it is well known that several molecular interactions exist between dye and protein molecule such as dye-water, protein-water (bound/trapped), dye-protein as well as water-water assemblies. Thus, our study depicts the clear picture of exact location of DCDAP dye in the protein (OVA) and the molecular interaction site in the absence of water molecules wherein the amino acids are involved in bonding. The first conformer (OVA-DCDAP 1) is highly stable with least B.E of -6.62 and LE of -0.29 whereas the last conformer (OVA-DCDAP 10) is least stable with B.E of -5.35 and LE of -0.23. Based on the outcome, 2^{nd},5^{th} and 6^{th} conformers (OVA-DCDAP 2, OVA-DCDAP 5 and OVA-DCDAP 6) bind similarly. Likewise, the 8^{th} and 10^{th} conformer OVA-DCDAP 8 and OVA-DCDAP 10 possess a similar binding pattern as that of OVA-DCDAP 1. Whereas, all other conformers, OVA-DCDAP 3, OVA-DCDAP 4, OVA-DCDAP 7 and OVA-DCDAP 9 docks at different sites in OVA.

As per the study, maximum number of interactions are found in OVA-DCDAP 2 conformer with total interaction of 17 while, the least number of interactions are found in OVA-DCDAP 3 conformer with 11 interactions (**Table 2**). OVA-DACDAP 4, OVA-DCDAP 6, OVA-DCDAP 9 and OVA-DCDAP 10 exhibits 16 bimolecular interactions. Similarly, 14 interactions exist in OVA-DCDAP 7 and OVA-DCDAP 8. Conformer OVA-DCDAP 1 and OVA-DCDAP 5 contains 15 and 12 of interactions respectively. Among the molecular interactions other interactions comprises of weak van der Walls forces which are found to be more predominant than the combined hydrogen bonding and hydrophobic interactions. This is clearly established from **Table 2** and **Figure S3.** wherein the energetically more stable conformers namely OVA-DACDAP 1-3 exhibit less than 2 HB interactions on the countering the other forces of attraction are found to be 7,10 and 9 respectively for the other set conformers.

Further, there exists a total of six distinct conformer structures which is represented in **Figure S2**. When analyzed, the molecular interactions of all 10 conformers, 1^{st}, 4^{th}, 5^{th} and 6^{th} (OVA-DCDAP 1, OVA-DCDAP 4, OVA-DCDAP 5 and OVA-DCDAP 6) are quite similar to each other even though these conformers have varying B.E as provided in **Table 1**. Similarly, the bimolecular interactions of OVA-DCDAP 2 and OVA-DCDAP 9 the same. There exist various intermolecular attractions between the DCDAP dye and the OVA protein such as (i) Hydrogen bonding that comprises of conventional as well as non-conventional HB of simply carbon hydrogen, (ii) Hydrophobic interactions that includes alkyl, pi-alkyl, pi-sigma, pi-anion, pi-lone pair, pi-cation, pi-donor and amide pi-stacked interactions. Further, other interactions such as weak van der Waals forces of attraction and unfavorable interactions are grouped under other interactions but, also contribute to the B.E of the conformers which in turn reflects the stability of the individual conformers.

The molecular interaction parameters existing between DCDAP dye and OVA is provided in the **Table 3** and the number of interactions of DCDAP dye with OVA is provided in **Table 2** and **Figure S3.** As per the computations, there exits more than one weak van der Waals forces of attraction in all conformers. Further, HB is also present in all conformers except for OVA-DCDAP 3. Alkyl and pi-alkyl hydrophobic interaction is present in all conformers except OVA-DCDAP 3 (consists of only alkyl interaction and not pi-alkyl interactions). Amide pi-stacked bonding and pi-donor bonding are only found with OVA-DCDAP 10 conformer. Similarly, pi-sigma hydrophobic interactions exist in OVADCDAP 2, OVADCDAP 4 and OVADCDAP 9 conformers. However, Pi-anion and

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Pi- cation interactions are visualized in OVA-DCDAP 4 and OVA-DACDAP 8 only. Interestingly pi-lone interaction is not common and is observed only in the 6th conformer. whereas, pi-cation is present only in OVADCDAP 8 conformer. And pi-lone pair hydrophobic interaction is noticed only with OVA-DCDAP 6 with bond distance of 2.96 nm.

Table 1: Energetics of 10 conformers of OVA-DCDAP

Conformer	Binding Energy (B.E)	Ligand efficiency	Inhibitory constant, Ki (mm)	Intermolecular energy	Intermolecular energy	Intermolecular energy
OVA-DCDAP 1	-6.62	-0.29	13.96	-7.52	-7.52	0
OVA-DCDAP 2	-6.49	-0.28	17.49	-7.38	-7.26	-0.12
OVA-DCDAP 3	-6.46	-0.28	18.44	-7.35	-7.31	-0.04
OVA-DCDAP 4	-6.37	-0.28	21.54	-7.26	-7.23	-0.03
OVA-DCDAP 5	-6.14	-0.27	31.54	-7.04	-6.86	-0.18
OVA-DCDAP 6	-5.94	-0.26	44.40	-6.83	-6.79	-0.04
OVA-DCDAP 7	-5.75	-0.25	61.32	-6.64	-6.65	0
OVA-DCDAP 8	-5.64	-0.25	74.04	-6.53	-6.49	-0.04
OVA-DCDAP 9	-5.60	-0.24	78.64	-6.49	-6.43	-0.06
OVA-DCDAP 10	-5.35	-0.23	119.79	-6.24	-6.27	0.02

Table 2: Number of interactions of DCDAP dye with OVA

Conformer	Hydrogen bonding	Hydrophobic Interactions	Other Interactions
OVA-DCDAP 1	1	7	7
OVA-DCDAP 2	2	5	10
OVA-DCDAP 3	0	2	9
OVA-DCDAP 4	3	6	7
OVA-DCDAP 5	1	5	6
OVA-DCDAP 6	2	7	7
OVA-DCDAP 7	2	6	6
OVA-DCDAP 8	1	7	6
OVA-DCDAP 9	5	6	5
OVA-DCDAP 10	4	7	5

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Table 3: Molecular interactions of DCDAP dye with OVA

Conformer	Binding energy	Hydrogen bonding	Bond Distances	Hydrophobic Interactions	Bond Distances	Other Interactions
OVA-DCDAP 1	**-6.62**	**C….H:** Glu 143	 3.04	**Alkyl:** Ala 149A Ala 153 Arg 345 Ile 145 Pro 144 **Pi-Alkyl:** Ala 149A Pro 144	 4.07 3.55 4.52 5.09 3.8 4.92 4.89	**van der Walls Forces** Asn 146 Glu 122 Glu 346 Gln 152 Gly 349 Thr 149 Val 347
OVA-DCDAP 2	**-6.49**	**C….H:** Glu 143 Glu 143	 3.04 3.25	**Alkyl:** Ile 145 Leu 156 **Pi-Alkyl:** Ala 149A Tyr 119 **Pi-Sigma:** Ile 145	 4.31 5.18 4.3 5.32 3.73	**van der Walls Forces** Asn 146 Arg 345 Gln 152 Glu 346 Gly 349 Lys 199 Pro 144 Thr 149 Val 347 Val 348
OVA-DCDAP 3	**-6.46**			**Alkyl:** Leu 138 Leu 327	 5.06 4.05	**van der Walls Forces** Ala 316 Ala 324 Asn 317 Glu 325 Ser 314 Ser 315 Ser 319 Ser 322 Ser 323
OVA-DCDAP 4	**-6.37**	**C….H:** Asn 47 Glu 214 Thr 211 **Conventional:** Leu 260	 3.42 3.33 3.15 1.95	**Alkyl:** Leu 260 Val 389 **Pi-Alkyl:** Pro 369 Val 210 **Pi-Sigma:** Val 389 **Pi-Anion:** Glu 214	 4.86 4.98 5.16 4.61 3.98 4.35	**van der Walls Forces** Arg 387 Glu 212 Glu 261 Gly 159 Ser 258 Ser 390 Val 251
OVA-DCDAP 5	**-6.14**	**C….H:** Glu 143 **Conventional:** Asn 146 Gly 349	 3.27 1.98 2.98	**Alkyl:** Ala 153 Ile 145 Pro 144 **Pi-Alkyl:** Ile 145	 3.26 4.16 4.4 5.45	**van der Walls Forces** Ala 149A Arg 345 Glu 122 Gln 152

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				Pro 144	4.41	Leu 156 Val 347
OVA-DCDAP 6	**-5.94**	**Conventional:** Thr 149	 2.93	**Alkyl:** Ala 149A Ala 153 Arg 345 Ile 145 Pro 144 **Pi-Alkyl:** Ala 149A **Pi-Lone pair:** Pro 144	 4.08 3.56 4.42 5.08 3.82 4.85 2.96	**van der Walls Forces** Asn 146 Gln 152 Glu 122 Glu 143 Glu 346 Gly 349 Val 349
OVA-DCDAP 7	**-5.75**	**Conventional:** Asn 101 Lys 105	 2.27 2.24	**Alkyl:** Lys 135 Lys 105 **Pi-Alkyl:** Arg 139 Lys 105 Lys 135 Pro 106	 4.82 3.94 4.51 4.98 5.46 4.88	**van der Walls Forces** Asp 108 Gly 140 Gly 141 Leu 142 Thr 104 Tyr 138
OVA-DCDAP 8	**-5.64**	**Conventional:** Lys 296	 2.04	**Alkyl:** Leu 41 Val 302 Val 302 **Pi-Alkyl:** His 44 His 44 His 45 **Pi-cation:** His 45	 4.17 4.34 4.43 4.49 4.02 5.08 2.2	**van der Walls Forces** Asn 298 Gln 174 Glu 40 Glu 295 Ser 301 Tyr 297
OVA-DCDAP 9	**-5.6**	**C….H:** Arg 345 Glu 122 **Conventional:** Asp 151 Gln 152 Lys 199	 3.39 2.97 2.97 2.06 2.96	**Alkyl:** Ile 145 Ile 145 Leu 156 **Pi-Alkyl:** Ala 149A Ala 149 **Pi-Sigma:** Val 347	 5.00 5.10 5.13 5.03 4.80 3.86	**van der Walls Forces** Ala 351 Asn 146 Glu 346 Gly 349 Val 348
OVA-DCDAP 10	**-5.35**	**C….H:** Ala 332 Gln 331 Gln 174 **Conventional:** Lys 296	 3.64 3.55 3.00 3.14	**Alkyl:** Val 333 Leu 173 **Pi-Alkyl:** His 45 Lys 296 Val 333 **Pi-Donor:** Lys 296 **Amide-Pi-stacked:** Tyr 298	 4.04 4.63 5.23 5.49 4.75 3.14 3.90	**van der Walls Forces** Asn 298 Val 172 Ser 177 Glu 294 Glu 295

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For OVA-DCDAP 1 and OVA-DCDAP 2 conformers, under HB only carbon-hydrogen (C…H) bond exist whereas, in OVA-DCDAP 4, OVA-DCDAP 5, OVA-DCDAP 9 and OVA-DCDAP 10 along with C…H bond there exits conventional hydrogen bonding (cHB). While, OVA-DCDAP 6 and OVA-DCDAP 7 have only cHB under the category of HB. Alkyl hydrophobic interaction is found in all ten protein-dye conformers. Except OVA-DCDAP 3, all other conformers contain pi-alkyl hydrophobic interaction. Pi-sigma interaction is present in 3 conformers which are, OVA-DCDAP 2, OVA-DCDAP 4 and OVA-DCDAP 9. Pi-anion interaction is present in OVA-DCDAP 4 and pi-cation interaction in OVA-DCDAP 8. Apart from these interactions, pi-lone pair, pi-donor and amide pi-stacked hydrophobic interactions are also involved in protein-dye conformers.

Binding site (BS) is the region on the protein that binds to another molecule or ligand (DCDAP dye) with specificity resulting in protein-dye interaction. The Mol.Doc studies of DCDAP dye with OVA protein indicates that there exist five distinct BS in OVA for DCDAP dye to reside. In all ten protein-dye conformers, out of the five BS in OVA at least one BS is preferred. Five different BS in OVA with respect to the residue obtained are presented in **Figure S4**. The location of dye in different domains of OVA are governed by the nature of interactions formed by the amino acid residues to the closer vicinity of the DCDAP molecule. As per the analysis, site 1 and site 5 are the most preferred binding site for DCDAP to reside in OVA whereas, site 3 is the least preferred and site. Interestingly, sites 2 and 4 are moderately preferred. The complete representation regarding the affinity of the individual conformers to various binding sites is provided as pictorial representation in **Figure3** and **Table S1**.

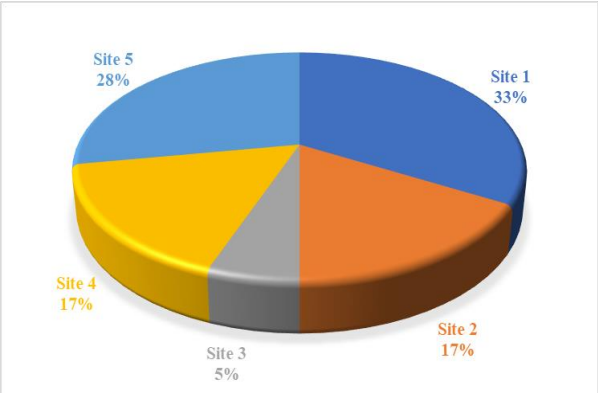

Figure 3: Pie chart representation on preferred binding site of OVA-DCDAP conformers

Various amino acids are involved in the interaction of all generated OVA-DCDAP conformers. Among them, polar amino acid, Asparagine (Asn) is predominantly involved whereas, Histidine (His) is the least involved in OVA-DCDAP complex. All polar amino acids are involved in any of the OVA-DCDAP conformers. Interestingly, the non-polar amino acids like phenylaniline (Phe), methionine (Met), tryptophan (Trp) and cysteine (Cys) are not involved in binding in any of the OVA-DCDAP conformer. However, valine (Val) is the most commonly involved non-polar amino acid followed by alanine (Ala), glycine (Gly) and leucine (Leu). In most of the protein-dye conformers, number of polar amino acids involved is greater than that of non-polar amino acids involved. **Figure 4** and **Table S2** depicts the graphical representation of the count of polar and non-polar amino acid involved in all the generated conformers. The count of respective amino acids is provided in **Figure 5** (for polar amino acid) and **Figure 6** (for non-polar amino acid). For OVA-DCDAP 1 and OVA-DCDAP 6 number of polar amino acid involved is same as that of number of non-polar amino acid. Whereas, only in OVA-DCDAP 5 the number of non-polar amino acid involved is slightly greater than that of number of polar amino acid involved. Both polar and non-polar amino acids are found in all ten generated OVA-DCDAP conformers. In order to establish the role of competing ligand influence on dye-protein binding, the flavonoids were docked with the most stable conformer (OVA-DCDAP 1). Through Mol.Doc approach, the binding of all ten conformations of DCDAP dye with OVA is depicted in **Figure 7**.

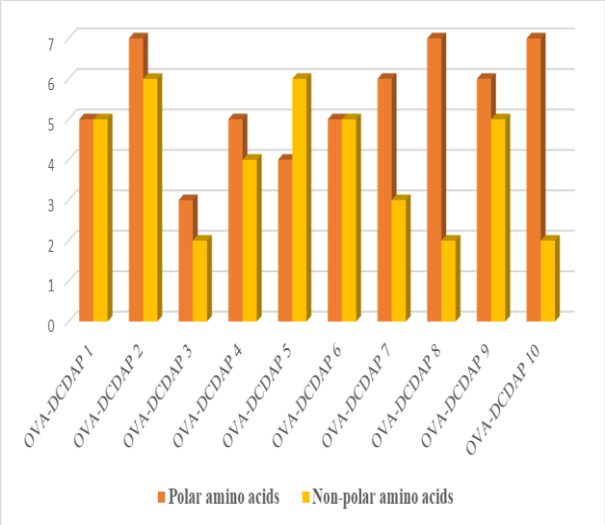

Figure 4: Graphical representation of Polar and non-polar Amino acids involved in OVA-DCDAP conformers

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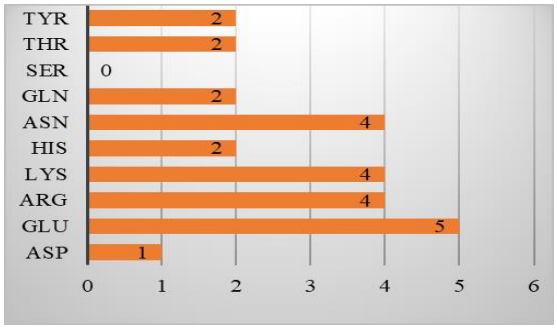

Figure 5: Count of Polar amino acids in OVA-DCDAP conformers

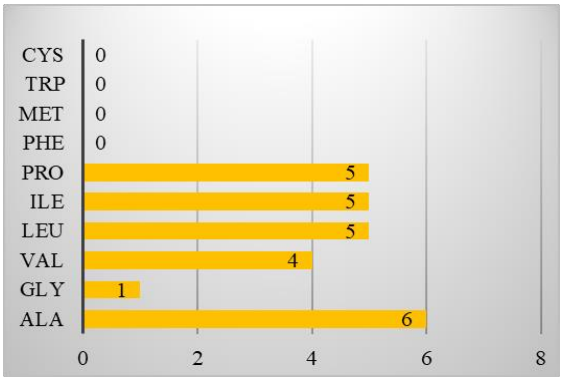

Figure 6: Count of non-polar amino acids in OVA-DCDAP conformers

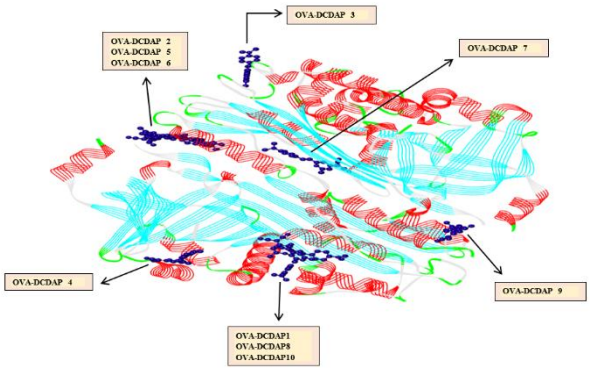

Figure 7: Ten conformations of DCDAP dye (depicted in blue colour ball and stick model) with three dimensional structures of OVA

Role of flavonoids on OVA-DCDAP1 conformer

The best conformer among the ten generated conformers with least binding energy, greater stability and greater intermolecular affinity is selected, which is OVA-DCDAP 1 represented in **Figure 8** is docked with various flavonoids to study its characteristics. The structure of DCDAP, OVA and flavonoids were ascertained on the basis of Lipinski rule and this method of formulating various energies associated with host-guest complexes. SMILES notation is elaborated in under the **Table S3**.

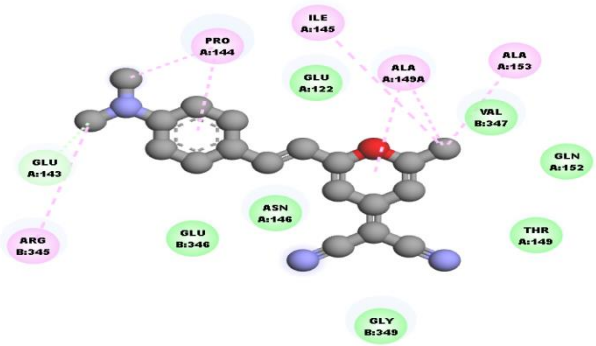

Figure 8: Best conformer of OVA-DCDAP (1st conformer – OVA-DDCDAP 1)

The stability of OVA-DCDAP complex increases when docked with flavonoids. For OVA-DCDAP docked with Apigenin(APG),Isorhamnetin (ISR), Gallocatechin(GCT), Epicatechin-3-gallate(ECG), and Leucocyanidin(LCD) the stability is comparatively lesser than that of OVA-DCDAP 1 conformer. B.E of OVA-DCDAP 1 is increased from -6.62 to -8.18 when Cyanidin(CYD) is introduced in protein-dye complex. B.E variation on docking OVA-DCDAP 1 with various flavonoids is provided in **Table 4**. For better understanding, the binding affinity of OVA-DCDAP 1 is taken as 100%, and the representation of Dye-Protein complex is provided as O-D when docked with Flavonoids. the variation of binding energy on docking OVA-DCDAP with various flavonoids is as shown in **Figure 9**. This ultimately reflects, when APG, ISR, GCT, ECG and LCD being docked with OVA-DCDAP, the complex is destabilized. Whereas, Catechin (CTC), Myricetin(MYC) and CYD is introduced in OVA-DCDAP 1 conformer, the molecule is highly stabilized.

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Table 4: Binding affinity of OVA-DCDAP

Molecular docking	Binding Energy (B.E)	Binding Affinity (%)
OVA-DCDAP	-6.62	100
O-D-PHR	-7.35	111
O-D-NRG	-7.96	120
O-D-APG	-6.13	92.6
O-D-LUE	-7.86	119
O-D-KMP	-7.99	121
O-D-QRC	-7.99	121
O-D-ISR	-6.31	95.3
O-D-MYC	-8.15	123
O-D-MRN	-7.97	120
O-D-CTC	-8.18	123
O-D-GCT	-4.83	72.0
O-D-EPC	-8.03	121
O-D-ECG	-4.37	66.0
O-D-LCD	-6.52	98.4
O-D -DPD	-7.93	120
O-D-CYD	-8.18	124

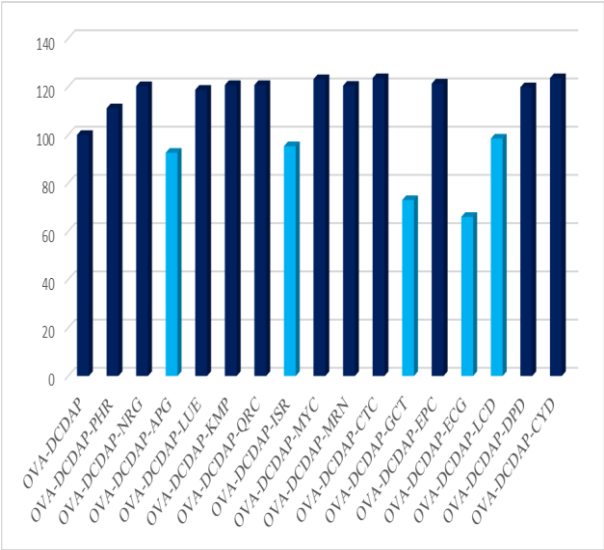

Figure 9: Graphical representation of binding affinity of OVA-DCDAP with various flavonoids

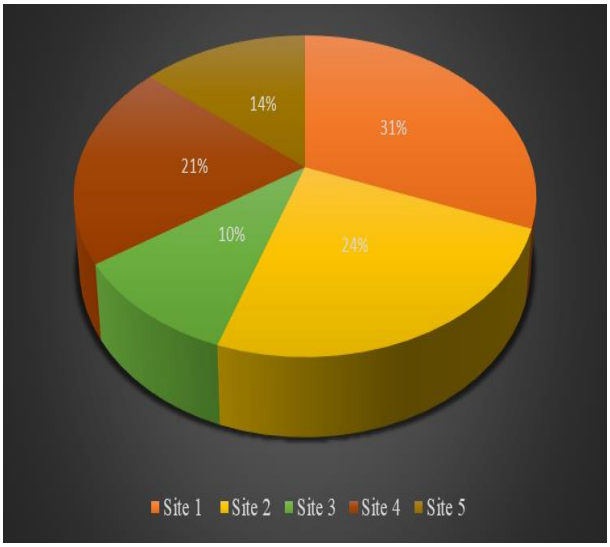

Figure 10: Pie chart representation on preferred binding site of OVA-DCDAP-Flavonoid

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On analyzing the data of molecular interactions, OVA-DCDAP 1 possesses 15 interactions out of which, only one interaction is attributed due to 14 other interactions are through hydrophobic and other weaker interactions. Other than ECG docked with the Protein-dye complex, no other flavonoid docked consists of unfavorable bonds. All Protein-Dye-Flavonoid complex consist of weak van der Waals forces of attraction. Protein-Dye have maximum number of interactions (count 21) when it is been docked with Kaempferon (KMP) and ISR whereas, have minimum interaction (count 10) when docked with Phloretin (PHR). OVA-DCDAP when docked with Naringenin (NRG), APG, Luteolin (LUE), Querctin (QRC) and Delphinidin (DPD) have interactions lesser than that of OVA-DCDAP 1 conformer. In most of the OVA-DCDAP-flavonoid complexes, number of hydrogen bonds (HB) involved are slightly greater than hydrophobic interactions. Whereas, in OVA-DCDAP molecule the number of hydrophobic interactions is much greater than the number of hydrogen bonds. O-D-ISR have maximum number of weak Van der Waals forces of attraction and O-D-LUE have the least number of weak wander Waals forces of attraction. **Table 5** provides the data on counts of various categories of attraction.

As per the information in **Table 6**, pi-alkyl hydrophobic bonds are visualized in all OVA-DCDAP-Flavonoid along with OVA-DCDAP molecule. Similarly weak Van der Waals forces of attraction is also found in all OVA-DCDAP-Flavonoid as well as in OVA-DCDAP molecule. Unfavorable donor-donor interaction of histidine (His 337) is found in O-D-ECG. Weak Van der Waals forces of attraction and unfavorable attractions are under the category of other interactions. At least one HB is available for all OVA-DCDAP molecule when combined with any Flavonoid. O-D-PHR and O-D-ECG contains pi-cation interactions. Alkyl hydrophobic interaction is found in O-D-PHR/GCT/EPC/ECG/ molecules. On the other hand pi-sigma bonds are found protein-dye complex is docked with NRG, APG, LUE, KMP, QRC and GCT. Pi-donor Hydrophobic interactions is found in O-D-APG molecule. Pi-anion hydrophobic interaction is contained in O-D-LUE and O-D-EPC molecules. O-D-ISR and O-D-CYD contains 5-lone pair hydrophobic interactions. When Myricetin is docked with OVA-DCDAP. There is a mild pi-stat hydrophobic interactions of TYR 138 is introduced.

5. Discussion

Almost all amino acids are involved when a flavonoid is docked with OVA-DCDAP molecule **Table 7**. Most frequently involved polar amino acids are glutamic acid (Glu), arginine (Arg), lysine (Lys), asparagine (Asn), glutamine (Gln) and serine (Ser). On the other hand, most frequently involved non polar amino acids are alanine (Ala), leucine (Leu) and Proline (Pro). Similarly, least frequently involved polar amino acids are histidine (His) and tyrosine (Tyr). Whereas methionine (Met) and tryptophan (Trp) are least frequently involved. Non polar amino acid cysteine (Cys) is not involved in any of the OVA-DCDAP molecule when docked with a flavonoid **Figure S5** and **Figure S6**. All OVA-DCDAP-flavonoid molecules contain at least one polar/non polar aminos.

KMP, MYC and LEU docked with OVA-DCDAP consists of maximum number of polar amino acids. Whereas ISR, LEU and CYD docked with OVA-DCDAP consists of maximum number of non-polar amino acids and DPD docked with OVA-DCDAP have least number of non-polar amino acids, In O-D-LEU maximum numbers of amino acids are involved.

In all OVA-DCDAP-Flavonoid molecules at least one binding site is preferred out of 5 binding sites in Ovalbumin (OVA) protein. Binding site 1 and site 2 are most preferred binding site whereas binding site 3 is the least preferred one **Figure 10**. Binding site 3 is only preferred site for O-D-LUE and O-D-DEL. Binding site 1 and site 5 are most preferred for O-D-QUE and O-D-CYL whereas binding site 2 and site 5 are preferred for MRN and EPC are docked with OVA-DCDAP. Three preferred binding sites, site 1 site 2 and site 3 are involved in ISR, CTC and LEU docked with OVA-DCDAP **Table 8**.

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Table 5: Details analysis and the bimolecular interactions existing due to docking of Flavonoid to OVA-DCDAP complex

Conformers	Binding energy	Hydrogen bonding	Bond Distance	Hydrophobic Interactions	Bond distances	Other Interactions
OVA-DCDAP	**-6.62**	Glu 143 (C...H)	3.04	**Alkyl:** Ala 149A Ala 153 Arg 345 Ile 145 Pro 144 **Pi-Alkyl:** Ala 149A Pro 144	 4.07 3.55 4.52 5.09 3.8 4.92 4.89	**van der Waals forces** Asn 146 Glu 122 Glu 346 Gln 152 Gly 349 Thr 149 Val 347
O-D-PHR	**-7.35**	Glu 264 Ser 258 Lys 42	1.97 2.02 2.11	**Pi-Alkyl:** Ala 46 Lys 42 **Pi-Cation:** Arg 387	 4.8 4.79 3.71	**van der Waals forces** Asn 47 Glu 261 Leu 60 Val 43
O-D-NRG	**-7.96**	Glu 339 Glu 339 Glu 294 Ile 168 Lys 292	1.9 2.04 2.36 2.5 2.28	**Pi-Alkyl:** Ile 168 **Pi-Sigma:** Ile 168	 5.33 3.84	**van der Waals forces** Arg 170 Asn 166 Gle 169 Gly 167 His 337 His 337 Lys 292
O-D-APG	**-6.13**	Asn 91 Asp 83 Gly 86 Ile 87D	2.05 2.09 1.90 3.06	**Pi-Alkyl:** Ile 87D **Pi-Sigma:** Ile 87D **Pi-Donor:** Gly 87A	 5.18 3.72 2.62	**van der Waals forces** Leu 84 Lys 83A Phe 87 Pro 85 Ser 94
O-D-LUE	**-7.86**	Ile 224 Lys 199 Lys 285 Tyr 222	2.18 1.97 2.6 2.23	**Pi-Alkyl:** Ala 353 Ala 353 Ala 357 Ile 224 Ile 224 Val 348 **Pi-Sigma:** Ala 357 **Pi-Anion:** Asp 202	 4.38 5.05 4.28 4.75 5.05 5.35 3.74 4.61	**van der Waals forces** Gln 223
O-D-KMP	**-7.99**	Asn 107 Asn 107 Gln 164 Leu 114 Phe 112 Thr 104 Tyr 110	1.82 2.13 2.77 1.84 2.95 2.23 2.25	**Pi-Alkyl:** Pro 106 Pro 106 **Pi-Sigma:** Pro 106	 4.84 4.96 3.76	**van der Waals forces** Ala 115 Arg 139 Gln 164 Lys 105 Lys 105

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						Phe 112 Ser 111 Ser 111 Ser 113 Thr 104 Tyr 110
O-D-QRC	**-7.99**	Glu 352 Gln 223	2.04 1.96	**Pi-Alkyl:** Lys 196 Val 348 **Pi-Sigma:** Val 348	 4.42 4.89 3.99	**van der Waals forces** Glu 122 Glu 346 Gly 225 Gly 349 Ile 224 Leu 226 Lys 283 Lys 283 Phe 227
O-D-ISR	**-6.31**	Ala 115(C….H) Asn 107 Gln 164 Gln 164 Phe 112 Phe 112 (C...H)	3.27 2.5 2.17 2.61 2.08 3.31	**Pi-Alkyl:** Pro 106 **Pi-Lone pair:** Asn 107	 4.89 2.87	**van der Waals forces** Arg 139 Asp 108 Gly 140 Leu 114 Pro 106 Ser 111 Ser 111 Ser 113 Ser 113 Ser 116 Thr 104 Thr 104 Trp 160
O-D-MYC	**-8.15**	Asp 108 Asp 108 Glu 136 Gly 140 Pro 106	2.15 2.55 2.13 2.12 1.71	**Pi-Anion:** Asp 108 **Amide Pi-Stacked:** Tyr 138	 3.69 5.07	**van der Waals forces** Arg 97 Arg 117 Arg 139 Arg 139 Arg 345 Asn 101 Asn 107 Leu 137 Lys 135 Ser 245A
O-D-MRN	**-7.97**	Arg 290 Arg 290 Lys 216 Lys 292 Pro 217 Pro 391	1.75 1.99 2.67 2.87 1.84 2.99	**Pi-Alkyl:** Arg 290 Arg 290 Val 218 **Pi-Anion:** Pro 391	 3.94 4.36 5.12 3.88	**van der Waals forces** Asn 341 Glu 339 Gln 219 Ile 340 Met 291 Pro 389
O-D-CTC	**-8.18**	Gln 164 Gln 164	1.81 2.10	**Alkyl:** Pro 106	 4.83	**van der Waals forces**

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		Gln 164 Asn 107 Phe 112 Ser 111 Leu 114 Thr 104	2.34 2.65 2.35 2.76 2.85 1.82	**Pi-Alkyl:** Pro 106 Pro 106	 5.30 5.38	Arg 139 Leu 114 Lys 105 Phe 112 Ser 111 Ser 113 Ser 113 Thr 104 Thr 165
O-D-GCT	**-4.83**	Asp 151 Val 178 Glu 101 Glu 101 Gln 152 Val 178	1.84 1.84 2.09 2.65 2.96 2.05	**Alkyl:** Ala 150 **Pi-Alkyl:** Ala 149A **Pi-Sigma:** Ala 150 Asp 151	 4.32 5.4 3.34 3.73	**van der Waals forces** Asp 179 Gln 148 Lys 199 Ser 176 Ser 179A
O-D-EPC	**-8.03**	Arg 290 (C...H) Glu 162 Gln 219 Lys 292 Pro 139	2.85 2.43 2.27 2.45 1.93	**Alkyl:** Lys 292 **Pi-Alkyl:** Arg 290 **Pi-Anion:** Glu 339	 4..55 4.33 3.42	**van der Waals forces** Arg 170 Asn 341 Met 291 Arg 170
O-D-ECG	**-4.37**	Glu 339 Gly 167 Glu 339 Glu 294 Lys 292	1.66 1.93 2.17 2.00 2.53	**Pi-Alkyl:** Lys 292 **Pi-Cation:** Arg 170	 5.30 4.05	**van der Waals forces** Arg 170 Asn 166 Asn 166 Gly 167 His 337 Ile 168 Lys 292
O-D-LCD	**-6.52**	Asn 107 Leu 114 Phe 112 Phe 112	2.82 2.39 1.93 2.04	**Alkyl:** Ala 115 Pro 106 **Pi-Alkyl:** Pro 106	 4.45 5.46 4.61	**van der Waals forces** Asp 108 Arg 139 Gln 164 Ilu 103 Lys 105 Pro 106 Ser 111 Ser 113 Ser 113 Ser 116 Thr 104 Trp 160
O-D-DPD	**-7.93**	Asp 356 Glu 363 Lys 283 Ser 361 Ser 359	2.12 2.33 2.39 2.15 2.82	**Pi-Alkyl:** Lys 283 Lys 283 Val 360 Val 360	 5.43 4.99 4.19 5.24	**van der Waals forces** Arg 282 Val 355
O-D-CYD	**-8.18**	Ala 149A Glu 122 Glu 143	2.03 2.98 1.88	**Pi-Alkyl:** Ala 149A Glu 122 **Pi-Lone pair:**	 4.41 3.86	**van der Waals forces** Ala 153 Arg 345

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				Pro 144	2.95	Asn 146 Gln 152 Glu 346 Gly 349 Ilu 145 Leu 156 Val 347 Val 348

Table 6: Number of interactions of OVA-DCDAP-Flavonoid

Type of complex	INTERACTIONS			
	Total	Hydrogen bonding	Hydrophobic	Others
OVA-DCDAP	15	1	7	7
O-D-PHR	10	3	3	4
O-D-NRG	14	5	2	7
O-D-APG	12	5	2	5
O-D-LUE	13	4	8	1
O-D-KMP	21	7	3	11
O-D-QRC	14	2	3	9
O-D-ISR	21	6	2	13
O-D-MYC	16	5	2	9
O-D-MRN	16	6	4	6
O-D-CTC	20	8	3	9
O-D-GCT	15	6	4	5
O-D-EPC	12	5	3	4
O-D-ECG	16	5	3	7+1 (UFB)
O-D-LCD	19	4	3	12
O-D-DPD	11	5	4	2
O-D-CYD	16	3	3	10

Table 7: Amino acids involved in OVA-DCDAP-Flavonoid

Molecule	Polar Amino acid	Non-Polar amino acid
OVA-DCDAP	Arg Glu	Ala Ile Pro
O-D-PHR	Arg Asn Glu Lys Ser	Ala Leu Val
O-D-NRG	Arg Asn Glu Lys	Gly Ile
O-D-APG	Asn Asp Lys Ser	Gly Ile Leu Phe Pro
O-D-LUE	Asp Gln Lys Tyr	Ala Ile Val
O-D-KMP	Arg Asn Gln Lys Ser Thr Tyr	Ala Leu Phe Pro
O-D-QRC	Gln Glu Lys	Gly Ile Leu Phe Val
O-D-ISR	Arg Asn Asp Gln Ser Tyr	Ala Gly Leu Phe Pro Trp
O-D-MYC	Arg Asn Asp Glu Lys Ser Tyr	Gly Leu Pro
O-D-MRN	Arg Asn Gln Glu Lys	Ile Met Pro Val
O-D-CTC	Arg Asn Gln Lys Ser Thr	Leu Phe Pro
O-D-GCT	Asp Glu Lys Gln Ser Thr	Ala Val
O-D-EPC	Arg Asn Gln Lys Ser Thr	Met Pro Val
O-D-ECG	Arg Asn Glu His Lys	Gly Ile
O-D-LCD	Arg Asn Asp Gln Lys Ser Thr	Ala Ile Leu Phe Pro Trp
O-D-DPD	Arg Asp Glu Lys Ser	Val
O-D-CYD	Arg Asp Glu Gln	Ala Gly Ile Lue Pro Val

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Table 8: Preferred binding site of OVA-DCDAP-Flavonoids

Conformer	Site 1	Site 2	Site 3	Site 4	Site 5
OVA-DCDAP				**X**	
O-D-PHR		**X**			
O-D-NRG	**X**				
O-D-APG			**X**		
O-D-LUE	**X**			**X**	
O-D-KMP	**X**				**X**
O-D-QRC	**X**	**X**		**X**	
O-D-ISR	**X**			**X**	
O-D-MYC		**X**			**X**
O-D-MRN	**X**	**X**		**X**	
O-D-CTC	**X**		**X**		
O-D-GCT		**X**			**X**
O-D-EPC		**X**			
O-D-ECG	**X**	**X**		**X**	
O-D-LCD			**X**		
O-D-DPD	**X**				**X**

6. Concluisons

The comparative studies between OVA-DCDAP versus OVA- DCDAP- Flavonoid(s) were ascertained energetically and based on the stability through molecular interactions.The maximum stability of OVA-DCDAP increases in the case of cyanidin and drastically decreases by docking of flavonoid (epicatechin-3-gallate). However, the total number of interactions between OVA-DCDAP conformer and that of OVA-DCDAP-flavonoid(s) almost remains the same. It is evident that the number of hydrogen bonding in OVA-DCDAP conformer is very few and even zero in the case of certain dye-protein conformer and consist of a greater number of hydrophobic interactions. Whereas, in OVA-DCDAP-flavonoid(s) the number of hydrogen bonding is greater than hydrophobic interactions. Unfavorable bonding prevails when OVA-DCDAP is docked with Epicatechin-3-Gallate. Thus, the OVA-DCDAP molecule is stabilized by hydrogen bonding while OVA-DCDAP-Flavonoid(s) are stabilised by hydrophobic interactions. Fewer number of amino acids are involved while in In OVA-DCDAP compared to OVA-DCDAP-flavonoid(s). Polar amino acids, Serine (Ser) is not involved in any of the OVA-DCDAP conformer but involved in 9 out of 16 OVA-DCDAP-flavonoid(s) molecules. Cysteine (Cys) is neither involved in any OVA-DCDAP conformer nor in OVA-DCDAP-Flavonoid(s). Non-polar amino acid Phenyl alanine (Phe) and Methionine (Met) and tryptophan (Trp) are not involved in any of the OVA-DCDAP conformers but involved when flavonoids are introduced in OVA-DCDAP 1 conformer. In OVA-DCDAP conformer and OVA-DCDAP-Flavonoid(s), binding site 1 is most preferred. Similarly, the least preferred binding site in OVA-DCDAP conformers and in OVA-DCDAP-flavonoid(s) is site 3. Wherein, Site 2 and Site 4 are better preferred when OVA-DCDAP docked with various flavonoids. The preference of binding in site 5 is reduced from 28% in OVA-DCDAP conformer to 14% in OVA-DCDAP-Flavonoid(s).

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SUPPORTING INFORMATIONS

Figure S 1: Structures of Flavonoids
Figure S 2: Distinct conformers of OVA-DCAP: (a) OVA-DCDAP 2; (b)OVA-DCDAP 9; (c)OVA-DCDAP 3; (d)OVA-DCDAP 7; (e)OVA-DCDAP 4; (f)OVA-DCDAP 1
Figure S 3: Interactions of DCDAP dye with OVA
Figure S 4: Five different binding sites in OVA with respect to residues
Figure S 5: Count of Polar amino acids in OVA-DCDAP-Flavonoid
Figure S 6: Count of non-polar amino acids in OVA-DCDAP-Flavonoid
Table S 1: Preferred binding site of OVA-DCDAP conformers
Table S 2: Amino acids involved in OVA-DCDAP conformers
Table S 3: SMILES notation of Flavonoids

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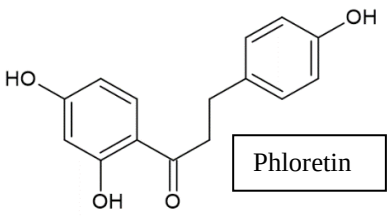

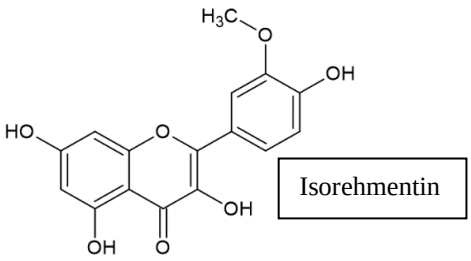

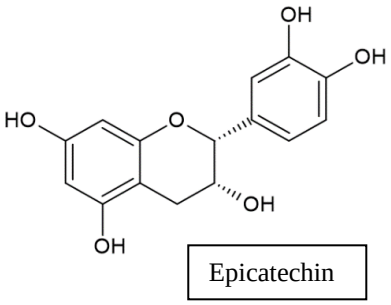

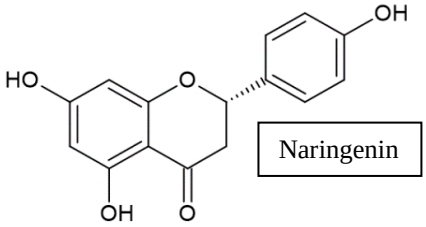

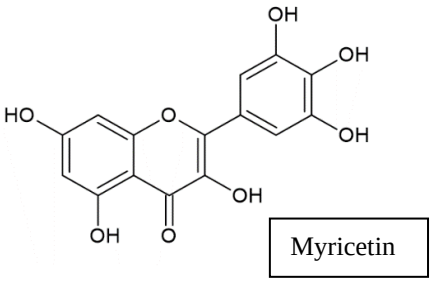

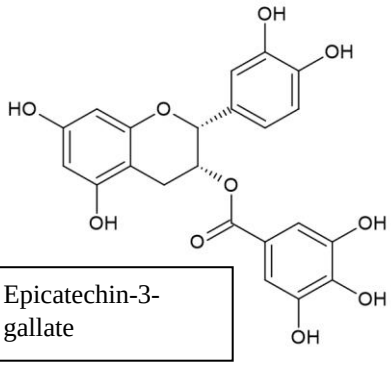

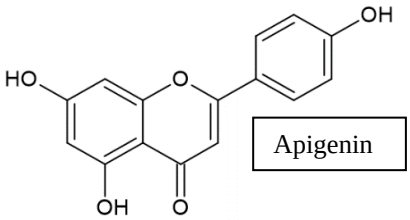

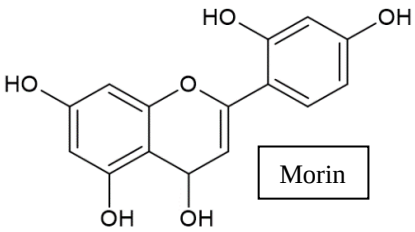

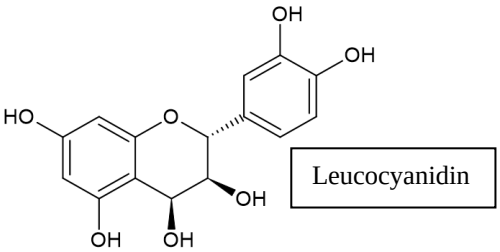

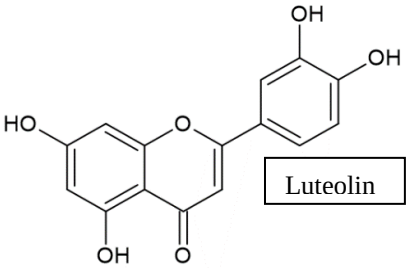

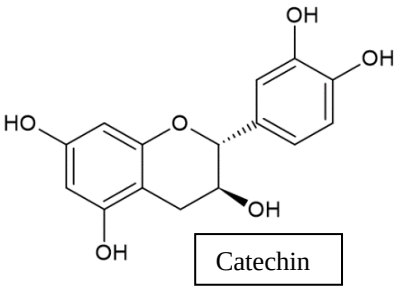

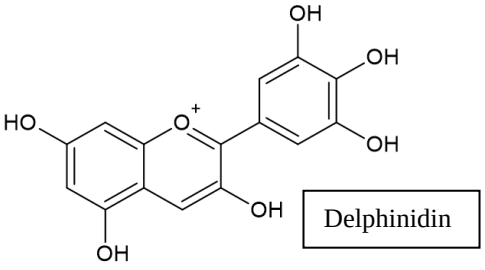

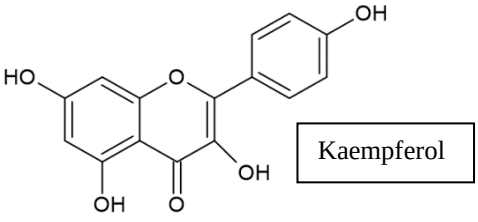

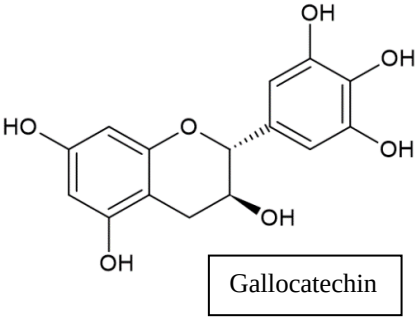

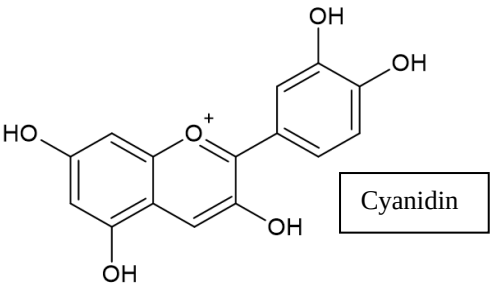

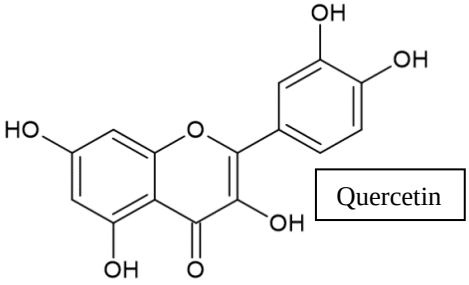

Figure S1: Structures of Flavonoids

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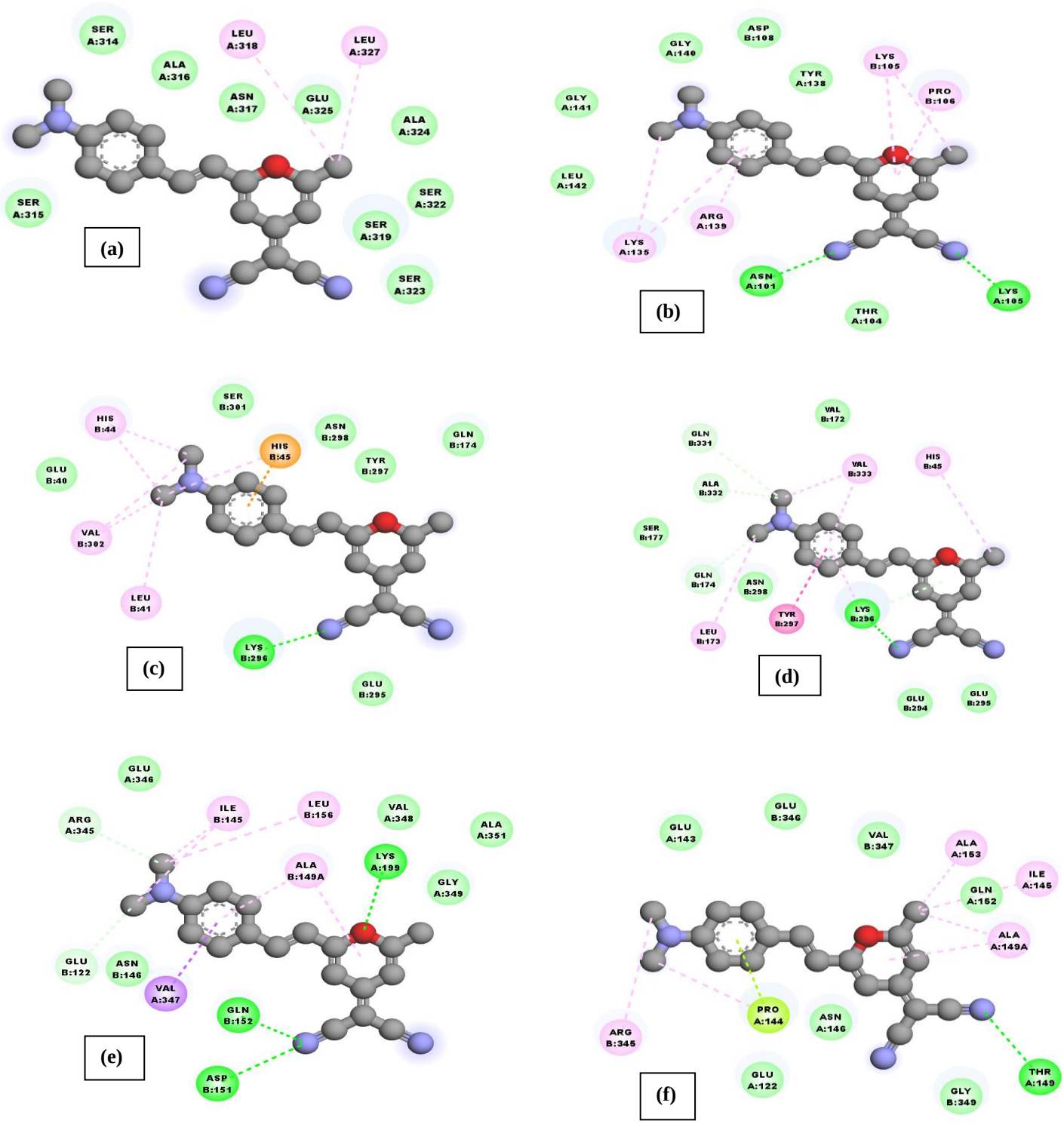

Figure S2: : Distinct conformers of OVA-DCAP: (a) OVA-DCDAP 2; (b)OVA-DCDAP 9; (c)OVA-DCDAP 3; (d)OVA-DCDAP 7; (e)OVA-DCDAP 4; (f)OVA-DCDAP 1

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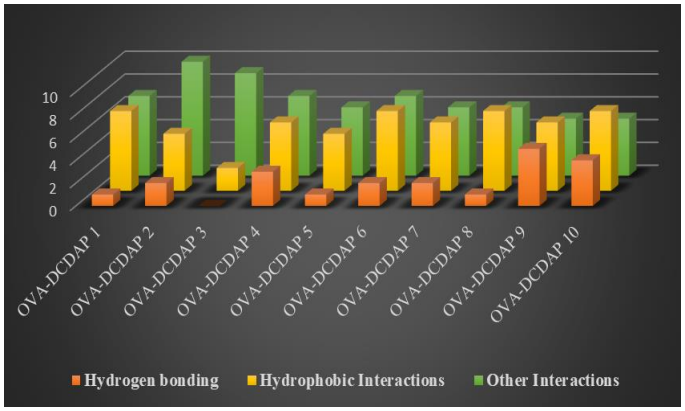

Figure S3: Interactions of DCDAP dye with OVA

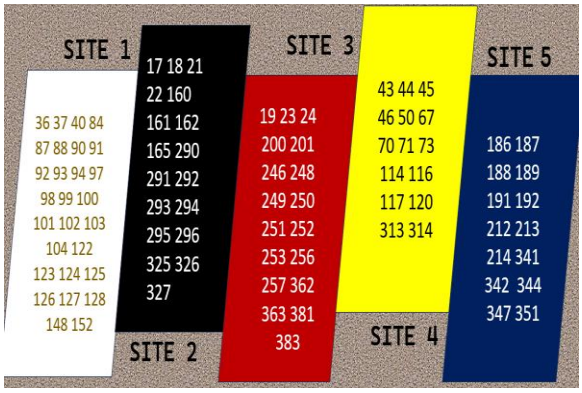

Figure S4: Binding sites residues in OVA

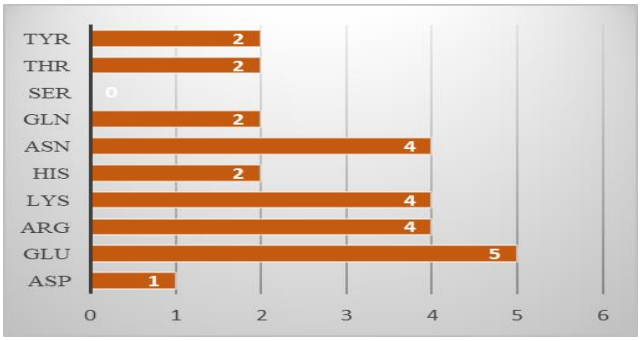

Figure S5: Count of Polar amino acids in OVA-DCDAP-Flavonoid

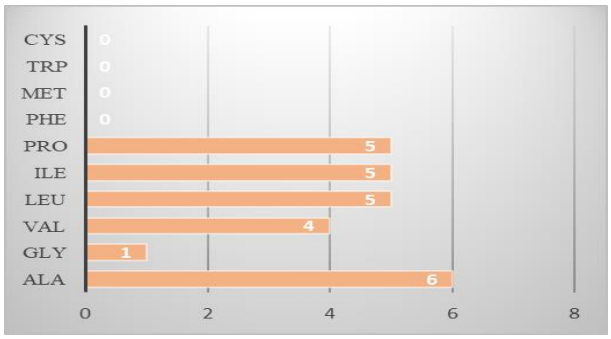

Figure S6: Count of non-polar amino acids in OVA-DCDAP-Flavonoid

Table S1: Preferred binding site of OVA-DCDAP conformers

Conformers	Site 1	Site 2	Site 3	Site 4	Site 5
OVA-DCDAP 1	**X**				**X**
OVA-DCDAP 2	**X**				**X**
OVA-DCDAP 3		**X**		**X**	
OVA-DCDAP 4			**X**		**X**
OVA-DCDAP 5	**X**				**X**
OVA-DCDAP 6	**X**				
OVA-DCDAP 7	**X**				
OVA-DCDAP 8		**X**		**X**	
OVA-DCDAP 9	**X**				**X**
OVA-DCDAP 10		**X**		**X**	

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Table S2: Amino acids involved in OVA-DCDAP conformers

Conformers	Polar	Non Polar
OVA-DCDAP 1	Glu Arg	Ala Ile Pro
OVA-DCDAP 2	Glu Tyr	Ala Leu Ile
OVA-DCDAP 3	Nil	Leu
OVA-DCDAP 4	Glu Asn Thr	Val Leu Pro
OVA-DCDAP 5	Glu Asn	Ala Gly Ile Pro
OVA-DCDAP 6	Arg Thr	Ala Ile Pro
OVA-DCDAP 7	Arg Lys Asn	Pro
OVA-DCDAP 8	Lys His	Val Leu
OVA-DCDAP 9	Asp Glu Arg Lys Gln	Ala Val Leu Ile
OVA-DCDAP 10	Lys His Asn Gln Tyr	Ala Val

Table S3: SMILES notation of Flavonoids

Flavonoids	TPS	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
Isorhamnetin	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