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Article

Computational analysis and in vitro investigation on Citrus flavonoids for inflammatory, diabetic and AGEs targets

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Flavonoids are a diverse class of polyphenolic substances largely found in plants including citrus peels and are reported to posess a variety of biological activities. We investigated important flavonoids apigenin, hesperidin, narigin, quercetin and tangeritine against diabetes and associated conditions. In current project drug likeness, ADMET analysis, molecular docking and *in vitro* assays were performed. The apigenin, quercetin and tanagretin exhibited compliance with Lipinski's rule of five. The molecular docking analysis showed best fit in transcriptional regulator 3TOP and 1IK3 in all tested compounds. During antioxidant assays, all flavonoids presented excellent activities. In the α-glucosidase assay, quercetin showed highest inhibition (76% at final concentration of 52 µg/ml) followed by tangeritin (73% at final concentration of 52 µg/ml). In case of 15-Lox assay, highest inhibition was seen in case of quercetin (75%) followed by apigenin (53%). In the AGEs assay, the quercetin showed 47% inhbition of protein cross link formation preceeded by the tenegretin exhited 37% inhibition. It was therefore concluded that tested flavonoids have significant activities in both *in silico* and *in vitro* models that is mainly due to differences in structural features and polar surface area.

Keywords: ADMET. In silico. Lipinski's rule. Flavonoids. Pharmacology.

INTRODUCTION

The diabetes mellitus is a metabolic disorder characterized by an increased in blood glucose levels and deficiency in secretion or action of insulin produced by the pancreas (Maritim, Sanders, Watkins 2003). The hyperglycemia plays a pivotal role in pathophysiology of diabetes like oxidative stress and abnormally elevated levels of lipids or lipoproteins in the blood (hyperlipidemia) causing dangerous complication (Kangralkar, Patil, Bandivadekar, 2010) including diverse micro (blindness, kidney disorder) and macro vascular (heart dysfunction, stroke) that can be fetal in patients with diabetes (Loghmani, 2005; Joshi, Parikh, Das, 2007).

It has been well documented that majority of diabetic complications (both micro and macro vascular) mainly occur due to advanced glycation end products (AGEs). These are heterogeneous molecules produced due to enzyme free (non-enzymatic) interaction of glucose (Glycation) with free sites of proteins, lipids, nucleic acids and amino groups (blood) (Aragno, Mastrocola, 2017). The AGEs are highly reactive florescent macromolecules and that disrupt various protein functions by acting on certain receptors RAGEs. Previous investigations had suggested that the development of advanced glycation

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end products is instigated and augmented when glucose levels are high or if there exist inflammation; thus playing a crucial role in this metabolic affiliation (Nowotny *et al*., 2015).

The flavonoids are diverse class of polyphenolic substances largely found in the plant families and more than 4000 different structure of flavonoids have been recognized (Kumar, Pandey, 2013). The flavonoids mainly impart color, give protection from fungal infections in fruits and vegetable and are famous because of useful health effects in humans (Peluso, 2006). The dietary flavonoids have protective function against coronary cardiovascular disease (Hertog, Feskens, Hollman, 1993) and possess antioxidant and biochemical effects linked with many disorders for instance Alzheimer's disease cancer, and atherosclerosis (Lee *et al*., 2009). They are also known as powerful inhibitors for various enzymes, like xanthine oxidase (XO), cyclo-oxygenase (COX), phosphoinositide 3-kinase, lipoxygenase (Walker *et al.,* 2000).

The citrus (Rutaceae) is one of the most popular world fruit crops that not only provides large amount of vitamin C, potassium, and pectin folic acid, but also is a rich source of various phenolics and flavonoids (Guimarães *et al*., 2009) including polymethoxylated flavonoids (PMFs). (Li *et al*., 2008). We therefore investigated citrus flavonoids using both *insilico* tools and *in vitro* assays to determine their biological potential regarding diabetes and allied co-morbidities.

MATERIAL AND METHODS

Chemicals and solvents

The chemicals and reagents used during the course of investigation were analytical grade. The enzyme α-glucosidase (*Saccharomyces cerevisiae*) and 15-lox (Glycine max) were purchased Sigma Aldrich (USA). The tested flavonoids tested were kindly provided by Prof. Dr. Luc Pieters, Natura, University of Antwerp, Belgium.

Drug likeness (Lipinski properties)

Using literature search, five most important and abundant flavonoids were selected for analysis including (1) apigenin, (2) hesperidin, (3) naringin, (4) quercetin and (5) tangeritin (Figure 1). The drug likeness or Lipinski properties (Lipinski *et al*., 1997) of all compounds was determined by using molinspiration tool (Hari, 2019).

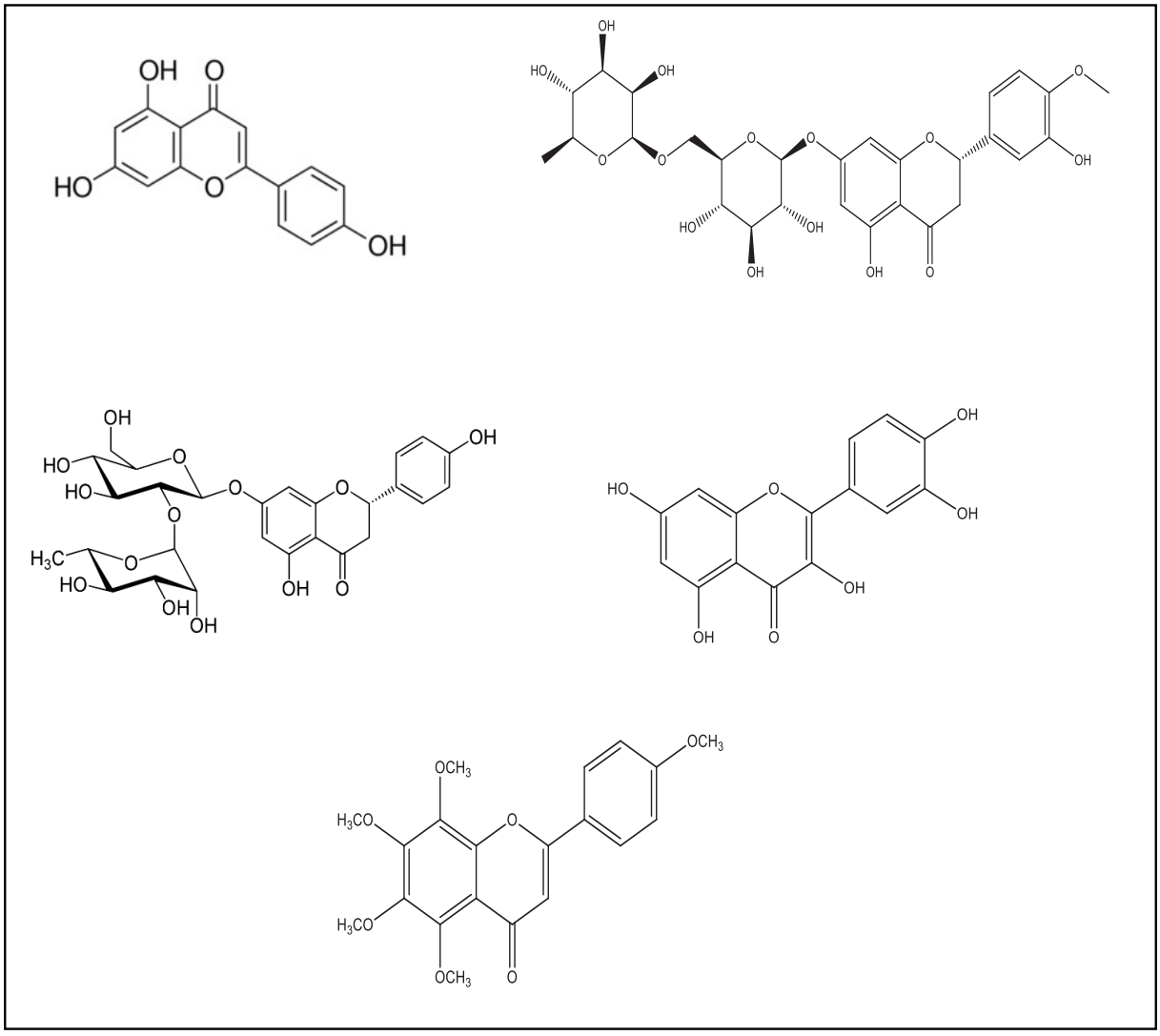

FIGURE 1 - Structure of flavonoids used for *in silico* and *in vitro* assays.

ADMET analysis

The ADMET analysis was performed using online tools including the SWISS ADME and pkCSM ADMET predictors (Han *et al*., 2019).

Molecular Docking

For Molecular docking studies, the X-ray crystallographic structures of the transcriptional regulators 3TOP (Ren *et al.,* 2011) and 1IK3 (Skrzypczak-Jankun *et al*., 2001) were obtained from the protein data bank (PDB). The active site dimensions for each protein were recorded by using their co-crystallized ligands. Then, the water molecules and co-crystallized ligand were removed and hydrogen atoms and charges were added. The SDF format for 3D structures of all the phyto-constituents were downloaded from Pubchem database and PDB files were generated in Discovery Studio Visualizer (2017). The molecular docking was performed using Lamarckian Genetic Algorithm embedded in AutoDock v 4.2. (Trott, Oslon, 2010). A total number of 45 poses were generated and clustered according to their RMSD values. Each cluster was carefully visualized in discovery studio visualizer (Berman *et al*., 2005) and putative binding modes were selected accordingly. Best docked structures based on the binding energy scores (**Δ**G) were chosen for further analyses. The hydrogen

bonding and hydrophobic interactions between ligand and protein were calculated by Discovery Studio Visualizer (2017) PYMOL and Ligplot+.

In vitro analysis

Antioxidant assays

The DPPH scavenging assay (Amin *et al*., 2016) and FRAP assay (Chandel *et al.,* 2020) were performed in order to determine the antioxidant potential.

Antidiabetic Assays

a) α-glucosidase inhibition assay

The α-glucosidase inhibition assay was performed by method adopted by Amin *et al*., (2016). Briefly, the plant material (various concentrations) or standard (acarbose) was incubated with α-glucosidase that extracted from *Saccharomyces cerevisiae* (0.2 U/mL in 0.1M phosphate buffer; pH 6.8) at 37°C for 10 min in 96 micro well plate. Then to this reaction (p-nitrophenyl-α-D-glucopyranoside) was added and placed in incubator up to 30 mint at 37°C. The % inhibition was calculated by using the below equation:

% Inhibition = 100 – (OD of test sample)/OD of control) x100

Advanced glycation end products Inhibition (AGEs assay).

Analysis of Cross-linked glycation

The cross linking percentage of the protein (cross-linked AGE's) were performed using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis (Elosta *et al.*, 2017).

Gel image analysis

The snapshots of gels were assimilated and analyzed using Gel doc system. And integrated density was calculated using ImageJ software (Ahmad, Pischetsrieder, Ahmed, 2007).

Anti-inflammatory activity

The anti-inflammatory potential of compounds was performed using 15-LOX (*Glycine max*) assay as explained earlier (Malterud, Rydland, 2000). Briefly, varying concentrations of test extract in DMSO was added to enzyme (200 U/mL) solution. The solution was incubated at room temperature (25°C) for 5 min. Finally, the absorbance was recorded instantaneously after the addition of substrate (linoleic acid in 0.2 M borate buffer, pH 9) after every minute up to 5 minutes at 234 nm by using UV spectrophotometer.

The enzyme inhibition activity was calculated as:

$$\% \text{ inhibition} = ([\Delta A1/\Delta t] - [\Delta A2/\Delta t] / (\Delta A1/\Delta t) \times 100$$

where $\Delta A1/\Delta t$ and $\Delta A2/\Delta t$ are the increase rate in absorbance at 234 nm for sample without test substance and with test substance respectively.

RESULTS AND DISCUSSIONS

Lipinski Rule

The "drug-likeness or Lipinski's rule of five (Lipinski *et al*., 1997) of the selected ligands was determined by using molinspiration tool (Hari, 2019). The Lipinski's rule of five is based on computational algorithms and is a very helpful tool that predicts the drug like properties of compounds. During our analysis, among all tested flavonoids, apigenin, quercetin and tanagretin exhibited drug like properties and showed compliance with Lipinski rule of five (Table I). However, hesperidin and naringin presented 3 violations, that are mainly because of their high molecular weight (>500KDa) and structural features. However they were still included in the further analysis as both of these compounds presented nice in vitro biological activities in earlier investigations (Amaro *et al.,* 2009; Parhiz *et al.*, 2015; Xiao, *et al*., 2018). Further, cyclic compounds and molecules with higher molecular weight tend to have such properties (like polar surface area,

high molecular weight, number of polar atoms etc) which make it difficult for conventional predictors to proceed for checking drug likeness of "drug-likeness," such as Lipinski's rule of five (Lipinski *et al*., 1997).

TABLE I - Lipinski properties of compounds

S.No	Compound	Molecular weight <500Da	Log p<5	H Bond Donor (5)	H-Bond acceptor <10	No of violations
1	**Apigenin**	270.2	2.46	3	5	0
2	**Hesperidin**	610.6	-0.55	8	15	3
3	**Naringin**	580.4	-0.37	8	14	3
4	**Quercetin**	302.3	1.68	5	7	0
5	**Tangeretin**	372.3	3.78	0	7	0

ADMET analysis

The pharmacokinetic parameter of ADMET (Absorption, distribution, metabolism, elimination and toxicity) of all tested flavonoids was performed using SWISS ADMET and pkCSM ADMET predictor. The SMILES of all flavonoids were downloaded from Pub chem and were used in further analysis. The ADMET features of all flavonoids are shown in Table II. The TPSA of all compounds was less than 100 that suggested good oral absorption or membrane permeability (Qidwai, 2016). However the hesperidin and Naringin were provided with much higher values, that indicated poor absorption. In case of lipophilicity, all compounds indicated nice lipophilic (AlogP98 ≤ 5) feature (Fonteh *et al*., 2015) with the exception of hesperidin and narigin. The CaCo-2 permeability, intestinal absorption (human), skin permeability and P-glycoprotein substrate or inhibitor are mainly used to predict the absorption level of the compounds. When the Papp coefficient is >8 x 10^{-6}, the predicted value is >0.90; thus, the compound has high CaCo-2 permeability and is easy to absorb. All compounds only apigenin and tangeretin showed high CaCo-2 permeability. Concerning the intestinal absorption (human), absorbance of less than 30% is thought as poorly absorbed. In our case all compounds showed excellent absorption except hesperidin and narigin. Likewise, for skin permeability, the compound with log Kp > -2.5, demonstrates a relatively low skin permeability. In case of all tested flavonoids, good skin permeability was recorded. P-glycoprotein is an associate of the ATP-binding transmembrane glycoprotein group [ATP-binding cassette (ABC)], that can excrete drugs or exogenous chemicals from cells. The distribution volume (VDss), Fraction unbound (human), CNS permeability and blood–brain barrier membrane permeability (logBB) are helpful tool to characterize the distribution of drugs in tissues. The case with VDss is lower than 0.71 L kg^{-1} (log VDss < -0.15), the distribution volume is considered to be relatively low. When VDss is higher than 2.81 L kg^{-1} (log VDss > 0.45), the distribution volume is considered to be relatively high. Our results showed that all compounds had low distribution volume (Table II).

For blood–brain barrier membrane permeability, the compounds with logBB >0.3 are considered to easily cross the blood–brain barrier easily. Since all of tested compounds has logBB <0.3; it suggested that all our compounds can't easily cross blood–brain barrier. For CNS permeability, compounds with logPS < -3 are not able to cross it. Based on this value, our compounds are unable to penetrate the CNS. In case of liver metabolism Cytochrome P450 based results indicated that none of compound can be metabolized in liver. During drug elimination, all of the compounds had low total clearance. Finally regarding toxicity; none of the compound was proven as toxic as prescribed in said criteria. Also all compounds were recoded as non-sensitive to skin (Table II).

TABLE II - ADMET properties of compounds

Properties	Compound				
	Apigenin	**Hesperidin**	**Naringin**	**Quercetin**	**Tangeretin**
TPSA (A°)	90.90	234.29 Å²	225.06	131.36	76.36
Consensus Log $P_{o/w}$	2.11	-0.72	-0.79	1.23	3.02
Absorption					
Water solubility (logmol/L)	-3.329	-3.014	-2.919	-2.925	-4.792
$CaCo_2$ permeability (log Papp in 10^{-6} cm/s)	1.007	0.505	-0.658	-.229	1.245
Intestinal absorption (human) (% absorbed)	93.25	31.481	25.796	77.207	98.478
Skin permeability (log Kp)	-2.735	-2.735	-2.735	-2.735	-2.678
P-Glycoprotein substrate	Yes	Yes	Yes	Yes	No
P-Glycoprotein I inhibitor	No	No	No	No	Yes
P-Glycoprotein II inhibitor	No	No	No	No	Yes
Distribution					
VDss (human, log L/kg)	0.822	0.996	0.619	1.559	-0.226
Fraction unbound (human) (Fu)	0.147	0.101	0.159	0.206	0.188
BBB permeability(logBB)	-0.734	-1.715	-1.6	-1.098	-1.026
CNS permeability (log PS)	-2.061	-4.807	-4.773	-3.065	-3.011
Metabolism					
CYP2D6 substrate	No	No	No	No	No
CYP3A4 substrate	No	No	No	No	Yes
CYP1A2 inhibitor	Yes	No	No	Yes	Yes
CYP2C19 inhibitor	Yes	No	No	No	Yes
CYP2C9 inhibitor		No	No	No	No
CYP2D6 inhibitor	No	No	No	No	No
CYP3A4 inhibitor	Yes	No	No	No	Yes
Excretion					
Total clearance (logml/min/kg)	0.566	0.211	0.318	0.407	0.78
Renal OCT2 substrate	No	No	No	No	No
Toxicity					
AMES toxicity	No	No	No	No	No
hERG I inhibitor	No	No	No	No	No
hERG II inhibitor	No	Yes	Yes	No	No
Hepatotoxicity	No	No	No	No	No
Skin sensitization	No	No	No	No	No

ADMET, absorption, distribution, metabolism, excretion, and toxicity; TPSA topological polar surface area; Consensus Log $P_{o/w}$ average of five different lipophilicities, Papp, apparent permeability coefficient; AMES, assay of the ability of a chemical compound to induce mutations in DNA; Kp, skin permeability constant; Fu, fraction unbound; BBB, blood–brain barrier; BB, blood–brain; CNS, central nervous system; PS, permeability-surface area; T. pyriformis, Tetrahymena pyriformis; LD, lethal dose; LOAEL, lowest-observed-adverse-effect level(Adopted from Han et al., 2019)

Docking Studies Using AutoDock Vina

After protein and ligand preparation, the docking was carried out using AutoDock Vina. The output files (PDBQT) were splitted and all poses (9 in total for each ligand) were visualized using Discovery studio. The docking scores and interactions types were recorded (Table III). The protein-ligand interactions were checked using PYMOL and Ligplot+. In the transcriptional regulator 3TOP, the Apigenin showed a nice fit in binding pocket with pose 6 with free binding energy -7.6 ΔG (kJ mol^{-1}) (Figure 2). The apigenin showed a strong H-bonding interaction with Glu 1400; Glu1397; Leu1291; Glu1284; Arg1333 and other interactions included Pi-sigma and vandervaal's interactions (Figure 3). Similarly, the hesperidin showed fitting in binding pocket of the protein with pose 3 with free binding energy -9.2 ΔG (kJ mol^{-1}) (Figure 2). The hesperidin showed a strong H-bonding interaction with various amino acid residues including Glu970; Val1993; Tyr967; Asp965 and other interactions included *pi-sigma* and vandervaal's interactions (Figure 2). Despite this molecule showed a violations in Lipinski's, rule, very strong interaction was recorded within putative binding site of protein, that predicts a strong inhibition of enzyme active site.

TABLE III - Docking score, H and non H-Bonding interactions of tested flavonoids

Compound	Binding free energy ΔG (kJ mol^{-1})	Pose rank	No of H bonds	H Bond Interaction Residues	Other interaction residues
3TOP					
Apigenin	-7.6	6	5	Glu 1400; Glu1397; Leu1291; Glu1284; Arg1333	Pro1329;Phe1289;thr1290
Hesperidin	-9.2	3	4	Glu970; Val1993; Tyr967; Asp965	Ala973;Asp969;Phe995;Pro994; Gly992; Ser 991; Trp1148; His1449; Arg1453;
Naringin	-7.9	1	5	Pro1445; His1443; Asn971; Asn977; Tyr769; Asp969	Ala1029
Quercetin	-7.8	7	7	Gln1 372; Arg 1377; Arg 1285; Gln 1286; Gln 1254; Ser1292; Asp1357	Val 1363; Asp 1281; Phe 1295
Tanagretin	-6.5	6	4	His1449;Lys1163;Thr1150; Asp1194	Leu1450;Glu1451His1149;Trp1148; Gly992
1IK3					
Apigenin	-7.1	7	4	Asp568; Arg 221; Asn218; Thr 443	His 219; Gly569;Arg580
Hesperidin	-7.8	7	4	Asp568, Arg221;Asn218; Thr443	His219;Gly569;Arg580;
Naringin	-9.1	1	6	Arg580;Asn218;Leu577 Thr445;Phe576;Gln574	Gln762;Ala442;Thr443;Thr581;Tyr 581;Trp 578;Gly579;Ile 440;
Quercetin	-9.7	1	6	Lys545;Phe162;Ala163; Val144;Asn146;Asp787	Pre549;Trp791;Asn164;Val539;Val540; His548;Asn788;
Tanagretin	-7.0	3	3	Tyr512;Arg386;Arg378	Asp428;Val589;Leu379;Pro432;Lys388 Asp431;Pro385

3TOP (crystral structure of The C-Terminal subunit of Human Maltase- Glucoamylase);1IK3 (Lipoxygenase-3 soyabean complex with 13(s) hydroperoxy-9(z),11(E)-octadecanoic acid.

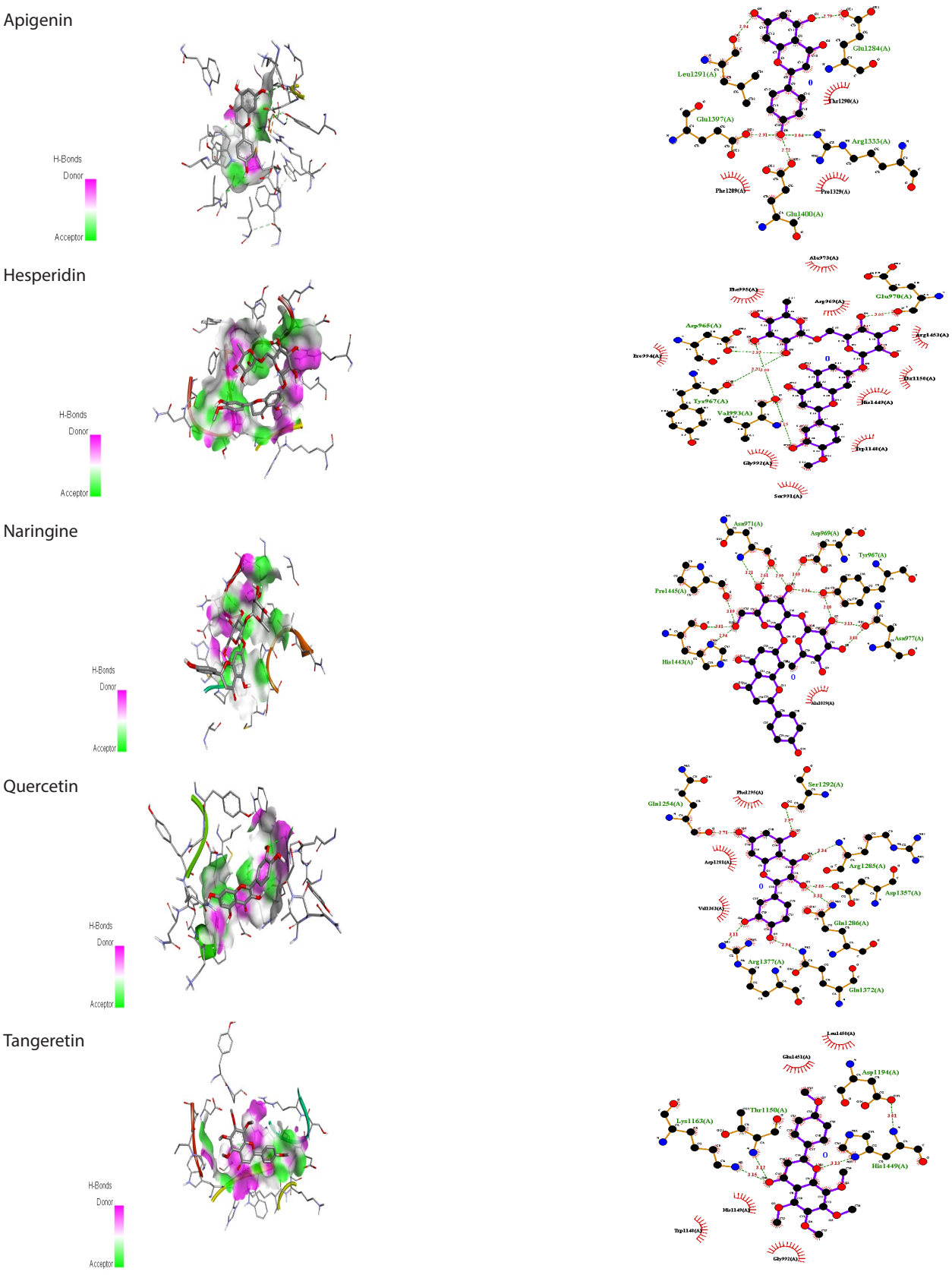

FIGURE 2 - 3D interaction and H, non-H Bonding interactions of flavonoids inside binding sites of transcriptional regulator **3TOP**.

In case of naringin, a good fitting in binding pocket of the protein with pose 1 and free binding energy -7.9 ΔG (kJ mol^{-1}) (Figure 2). The naringin presented a strong H-bonding interaction with diverse amino acid residues including Pro1445; His1443; Asn971; Asn977; Tyr769; Asp969 and other interactions included *pi-sigma* and vandervaals interactions (Figure 2). Although, naringin also violated Lipinski's rule; very strong interaction was recorded within putative binding site of protein, that also predicts a strong inhibition of enzyme active site. On the other hand quercetin presented nice fitting in binding pocket of the protein with pose 7 and free binding energy -7.8 ΔG (kJ mol^{-1}) (Figure 2). The quercetin presented a strong H-bonding interaction with diverse amino acid residues including Gln1 372; Arg 1377; Arg 1285; Gln 1286; Gln 1254; Ser1292; Asp1357 and other interactions included *pi-sigma* and vandervaal's interactions (Figure 2). Finally in case of tenegretin, pose 6 (-6.5 ΔG (kJ mol^{-1}) showed best fitting with putative binding site of 3TOP (Figure 2). The ligand presented a good H-bonding interaction with amino acid residues including His1449; Lys1163; Thr1150; Asp1194 and other interactions included *pi*-sigma and vandervaal's interactions.

In the transcriptional regulator 1IK3, the apigenin showed a nice fit in binding pocket with pose 7 with free binding energy -7.1 ΔG (kJ mol^{-1}) (Figure 3). The apigenin showed a strong H-bonding interaction with Asp568; Arg 221; Asn218; Thr 443 and other interactions included Pi-sigma and vandervaal's interactions. Similarly the hesperidin showed fitting in binding pocket of the protein with pose 7 with free binding energy -7.8 ΔG (kJ mol^{-1}) (Figure3). The hesperidin showed a strong H-bonding interaction with various amino acid residues including Asp568, Arg 221; Asn218; Thr443 and other interactions included *pi-sigma* and vandervaal's interactions (Figure 3). Despite this molecule showed a violations in Lipinski's, rule, a strong interaction was recorded within putative binding site of protein, that predicts a strong inhibition of enzyme active site.

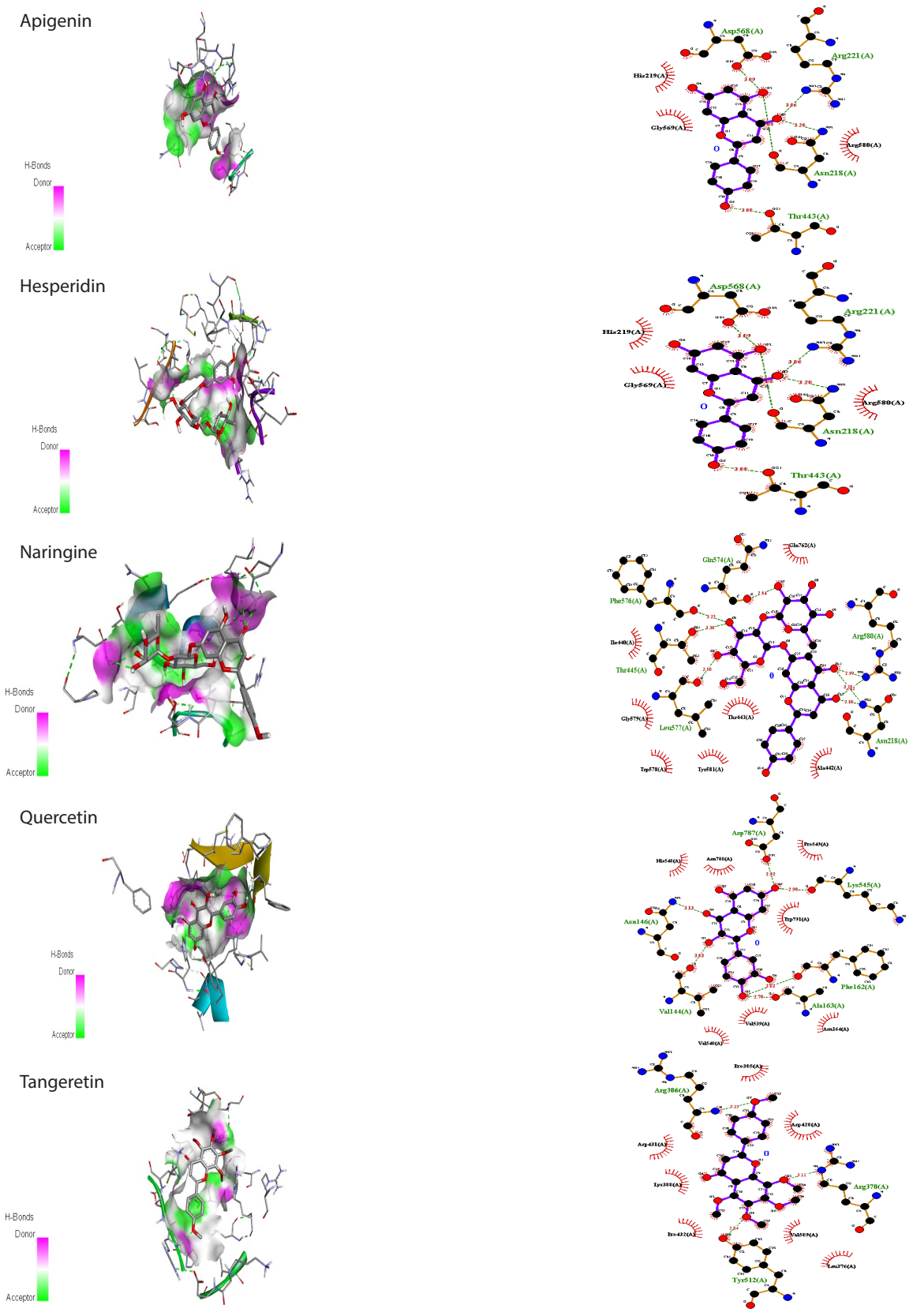

FIGURE 3 - 3D interaction and H, non-H Bonding interactions of flavonoids inside binding sites of transcriptional regulator **1IK3**.

In case of naringin, a good fitting in binding pocket of the protein with pose 1 and free binding energy -9.1 ΔG (kJ mol^{-1}) (Figure 3). The naringin presented strong H-bonding interaction with diverse amino acid residues including Arg580; Asn218; Leu577 Thr445; Phe576; Gln574 and other interactions included *pi*-sigma and vandervaal's interactions. Although, naringin also violated Lipinski's rule; very strong interaction was recorded within putative binding site of protein, that also predicts a strong inhibition of enzyme active site. On the other hand quercetin presented nice fitting in binding pocket of the protein with pose 1 and free binding energy -9.7 ΔG (kJ mol^{-1}) (Figure 3). The quercetin presented a strong H-bonding interaction with diverse amino acid residues including Lys545; Phe162; Ala163; Val144; Asn146; Asp787 and other interactions included *pi-sigma* and vandervaal's interactions. Finally in case of Tangeretin, pose 3 (-7.0 ΔG (kJ mol^{-1}) (Table II) showed best fitting with putative binding site of 3TOP (Figure 3). The ligand presented a good H-bonding interaction with amino acid residues including Tyr512; Arg386; Arg378 and other interactions included *pi*-sigma and vandervaal's interactions.

In vitro Assays

Initially all flavonoids were screed for their antioxidant activities using two different mechanism i.e DPPH and FRAP. During antioxidant assay, all flavonoids presented excellent activities (Table IV), that is due to flavone backbone and attached OH groups (Kumar, Mishra, Panday, 2013). The occurrence of strong antioxidant potential od flavonoids makes them ideal candidate for various biological activities as reported earlier (Panday, Mishra, Mishra, 2012). Based on our findings, we processed all flavonoids for further activities.

TABLE IV - Antioxidant activity of tested flavonoids

Sample	DPPH	FRAP
	(IC_{50} µg/mL)	(µM)
Tangeretin	177.0	11.56
Quercetin	1.01	55.60
Hesperidin	14.5	226.45
Apigenin	109.5	21.24
Naringin	122.1	24.01
Reference standard	0.52 [3]	-

[1] mg equivalents per gram of gallic acid, [2] mg equivalents per gram of rutin, [3] Rutin (µg/mL),

AGEs inhbition, α-glucosidase and 15-Lox assays

The flavonoids were tested for the inhibition of α-glucosidase. Among all, quercetin showed highest inhibition (76% at final concentration of 52 µg/ml) followed by tangeritin (73% at final concentration of 52 µg/ml). Unexpectedly, hesperidin showed slight inhibition (37% at final concentration of 52 µg/ml), that shows its least contribution towards inhibition of enzyme (Table V). It is important to note that hesperidin violated the Lipinski's rule, however during docking studies, the highest binding affinity was recorded (-9.2 ΔG (kJ mol^{-1}). This could possibly be due to fact that since the drug molecule is not following completely the drug likeness rule due to its large surface area, it may bind to another site within enzyme moiety. Likewise, naringin also presented least inhibition (17%) at tested concentration. This could possibly due to above stated reason.

TABLE V - AGEs, α-glucosidase and 15-Lox inhibition assay of tested Flavonoids

Compound	Assay		
	BSA-fructose IC_{50} (mg/ml)	α-glucosidase % inhibition (µg/ml	15-Lox % inhibition
Apigenin	31%	59	53
Hesperidin	28%	37	18
Naringin	30%	17	17
Quercetin	47%	76	75
Tangeretin	37%	73	inactive
Standard	0.04[1]	53[2]	56[3]

[1] rutin [2] Acarbose 0.25 mM), [3]quercetin (at 1.25ug/ml final concentration)

In case of 15-Lox assay, highest inhibition was seen in case of quercetin (75%) followed by apigenin (53%). These findings are in accordance with molecular docking results. Further all other compounds were only slightly active (Table V).

During AGEs experiments non-oxidative cross link inhbition assays were performed. The tanagretin and quercetion were selected for SDS-PAGE analysis (Figure 4). The developed gel was stained with coomsie brillient blue and images were obtained using Gel Doc systems. Finally ImageJ tool was used for detrmination of integrated density (IntDen) (Table VI). The integrated density was employed further for detrmination of % inhbition. The tenegretin exhited 37% inhibition at the tested concentration wheras the quercetin was able to show 47% inhbition. It was thus concluded that quercetion is better inbitor of protein cross link formation compared to tangertin. All other flavonoids were not tested due to limited avaliability of chemicals.

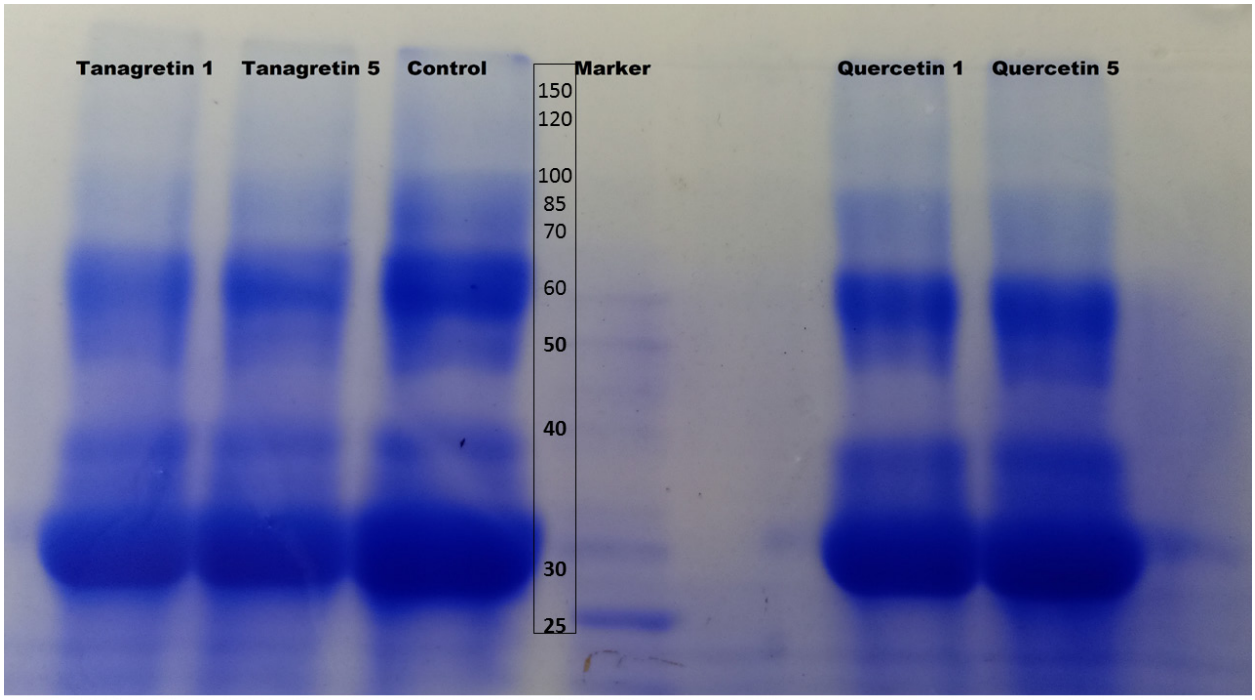

FIGURE 4 - Gel Dock analysis of cross linked AGEs.

TABLE VI - Determination of integrated density using Image J tool

Compound	Area	Mean	Stedev	Min	Max	Intden	Median	Raw Intden
Tanegretin-1	79560	112.973	7.285	86	136	8988155	114	8988155
Tanegretin -2	85680	95.722	7.615	73	121	8201503	95	8201503
Control-3	179820	72.699	7.566	52	108	13072688	71	13072688
Quercetin -1	96768	73.252	6.076	56	101	7088462	73	7088462
Quercetin-2	100224	68.968	5.197	54	94	6912231	68	6912231

Stdev=standard deviation, Intdev=integrated density

CONCLUSION

It was concluded that tested flavonoids have significant activities against tested models both *in silico* and *in vitro*. The flavonoids from diverse compound classes have different mode of fitting in active pockets of target proteins that is mainly based on the structural features and polar surface area.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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