Deciphering inhibitory activity of flavonoids against tau protein kinases: A coupled molecular docking and quantum chemical study

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Abstract

Today, Alzheimer's disease (AD) is one of the most important neurodegenerative disorders that affected millions of people around the world. Hundreds of academic investigations highlighted the potential roles of natural metabolites in the cornerstone of AD prevention. Nevertheless, alkaloids are only metabolites that successfully showed promising clinical therapeutic effects on the prevention of AD. In this regard, other classes of plant metabolites such as flavonoids are also considered to be promising substances in the improvement of AD complications. The lack of data on molecular mode of action of flavonoids inside brain tissues, and their potential to transport across the blood-brain barrier, a physical hindrance between bloodstream and brain tissues, limited the large-scale application of these compounds for AD therapy programs. Herein, a coupled docking and quantum study was applied to determine the binding mode of flavonoids and three protein kinases involved in the pathogenesis of AD. The results suggested that all docked metabolites showed considerable binding affinity to interact with target receptors, but some compounds possessed higher binding energy values. Because docking simulation cannot entirely reveal the potential roles of ligand substructures in the interaction with target residues, quantum chemical analyses (QCAs) were performed to cover this drawback. Accordingly, QCAs determined that distribution of molecular orbitals have a pivotal function in the determination of the type of reaction between ligands and receptors; therefore, using such quantum chemical descriptors may correct the results of virtual docking outcomes to highlight promising backbones for further developments.

***KEYWORDS*:** Docking, Quantum calculations, Flavonoids, Kinase, Alzheimer.

ABBREVIATIONS:

AD: Alzheimer's disease; **Aβ**: amyloid-β; **τ**: tau; **ERK1/2**: extracellular-regulated kinase 1/2; GSK-3β: glycogen synthase kinase-3β; **Cdk5**: cyclin-dependent kinase 5; **PKC**: protein kinase C alpha; **PKB/AKT**: protein kinase B; **PKA**: protein kinase A; **DYRK1A**: dual-specificity tyrosine-[Y]-phosphorylation-regulated kinase 1A; **CK1d**: casein kinase 1 delta; **MAPK10**: Mitogen-activated protein kinase 10; **JNK3**: c-Jun N-terminal kinase 3; **P38**: p38 delta kinase; **BBB**: the blood-brain barrier; **FMO**: frontier molecular orbitals; **HOMO**: highest occupied molecular orbital; **LUMO**: lowest occupied molecular orbital; **FERMO:** Frontier Effective-for-Reaction Molecular Orbital; **HSAB**: hard-soft acid-base; **KS:** Kohen-Sham; **HF**: Hartree–Fock; **DFT:** Density functional theory; **C3G**: Cyaniding-3-O-glucoside; **M3G**: malvidin-3-O-glucoside; **AChE**: acetylcholinesterase; **MEP:** Molecular electrostatic potential; **QM:** quantum mechanics; **HSAB:** Pearson's hard-soft, acid and base; **CNS:** Central nervous system.

1. INTRODUCTION

"*I have lost myself*" has sorrowfully expressed by *Auguste Deter,* the first patient diagnosed with AD (Maurer *et al.*, 1997), and that was a story to set off research on this brain disease. Simply, AD targets innermost tissues and/or signaling pathways of the brain, consequently leading to cognitive dysfunction, dementia, depression, and degeneration of brain cells (Dubois *et al.*, 2018, McDade and Bateman, 2017). Although the molecular basis of AD pathogenesis is not comprehensively understood, the accumulating body of evidence suggests that the disease mainly causes damage to brain cerebral cortex and hippocampus regions, by which the injuries gradually distend to other parts of the brain (Dubois *et al.*, 2018).

The hyper-phosphorylation of τ protein, the aggregation of Aβ fibrils, and enhanced oxidative stress are three characteristic hallmarks of AD affected brains (Association, 2018, Maurer *et al.*, 1997). Microtubule-associated τ protein is widely expressed in neurons, involved in the stabilization of neuronal scaffold, has structurally four domains to interact with related cellular targets, and is phosphorylated at 85 important amino acid sites (Martin *et al.*, 2013). Functionally, some transferase enzymes are responsible to substitute a phosphate moiety from energy donor substances such as ATP and GTP to specific biomolecules or substrates, which are involved in the phosphorylation of τ protein (Martin *et al.*, 2013). Various types of τ protein kinases have been identified and their function, structure, regulation and involvement in neurodegenerative processes linked to Alzheimer pathology are well described (Chico *et al.*, 2009). In this regard, protein kinases including ERK1/2 (Sun and Nan, 2017), GSK-3β (Lescot *et al.*, 2005), Cdk5 (Liu *et al.*, 2016), PKC (Clark *et al.*, 1991), PKB/AKT (Griffin *et al.*, 2005), PKA, DYRK1A, CK1d, MAPK10, JNK3 and p38 (Alvarez *et al.*, 1999, Dolan and Johnson, 2010, Schwab *et al.*, 2000) are deemed to be dysregulated and involved in the hyperphosphorylation of τ protein .

Some factors including low bioavailability, ability to transport across BBB (Sweeney *et al.*, 2018), and possible side effects of natural products in brain tissues are limited their widespread application in AD therapy horizons (Scotti and Scotti, 2018). Up to now, among natural products, alkaloids are only pioneer metabolites in the cornerstone of human diseases prevention (Ng *et al.*, 2015, Rasouli *et al.*, 2020). Additionally, several flavonoids have also transported across the BBB to interact with a variety of brain proteins and signaling pathways

(Faria *et al.*, 2014, Yang *et al.*, 2014), but their molecular mode of action remained unknown (Faria *et al.*, 2012).

Studies demonstrate that data obtained from docking results might determine the physical interaction of ligands-receptors mediated by sharing electrons from each side of reaction (Glaab, 2015, Rasouli *et al.*, 2017). In such a situation, determining the portion of each ligand moiety or residue side chain in the debuting of expected interactions is difficult or not fundamentally possible (Rasouli *et al.*, 2017). On the other hand, current docking scoring functions could not effectively highlight the priority of ligands atoms or active site residues in the first encounter of ligand-receptor (Klebe, 2006).

Over the past decades, molecular docking simulation has adequately been helping researchers to know how ligands correctly interact with their targets (Rasouli *et al.*, 2017). Despite its popularity, the molecular docking method presently suffered from several limitations including the lack of qualitative databases for validating the outcomes, the presence of false-positive results, and the obscurity linked to docking scoring mathematical functions (Glaab, 2015, Klebe, 2006). To solve these problems, studies suggested that docking results alone are not reliable, and coupling these outcomes with advanced computational methods such as molecular dynamics simulation and quantum chemical analyses might partly overcome these concerns and improve fidelity and accuracy of docking outputs (Ghazvini *et al.*, 2018, Raha and Merz, 2004, Rasouli *et al.*, 2017, Yuriev and Ramsland, 2013). To evaluate this possibility, quantum chemical calculations and traditional docking simulation were coupled for different targets and the results are as the follows.

2. COMPUTATIONAL DETAILS

2.1. LIGAND PREPARATION

Because of limitations with measuring flavonoids mode of action within the brain tissues, the fingerprint of flavonoids appearance in the central nervous system has only beeen reported by limited number of studies (Faria *et al.*, 2012, Faria *et al.*, 2014, Faria *et al.*, 2010, Ferri *et al.*, 2015, Yang *et al.*, 2014). Accordingly, 15 flavonoid metabolites with potential to transport across the BBB were chosen as input ligands for computational analyses (***Fig.1***).

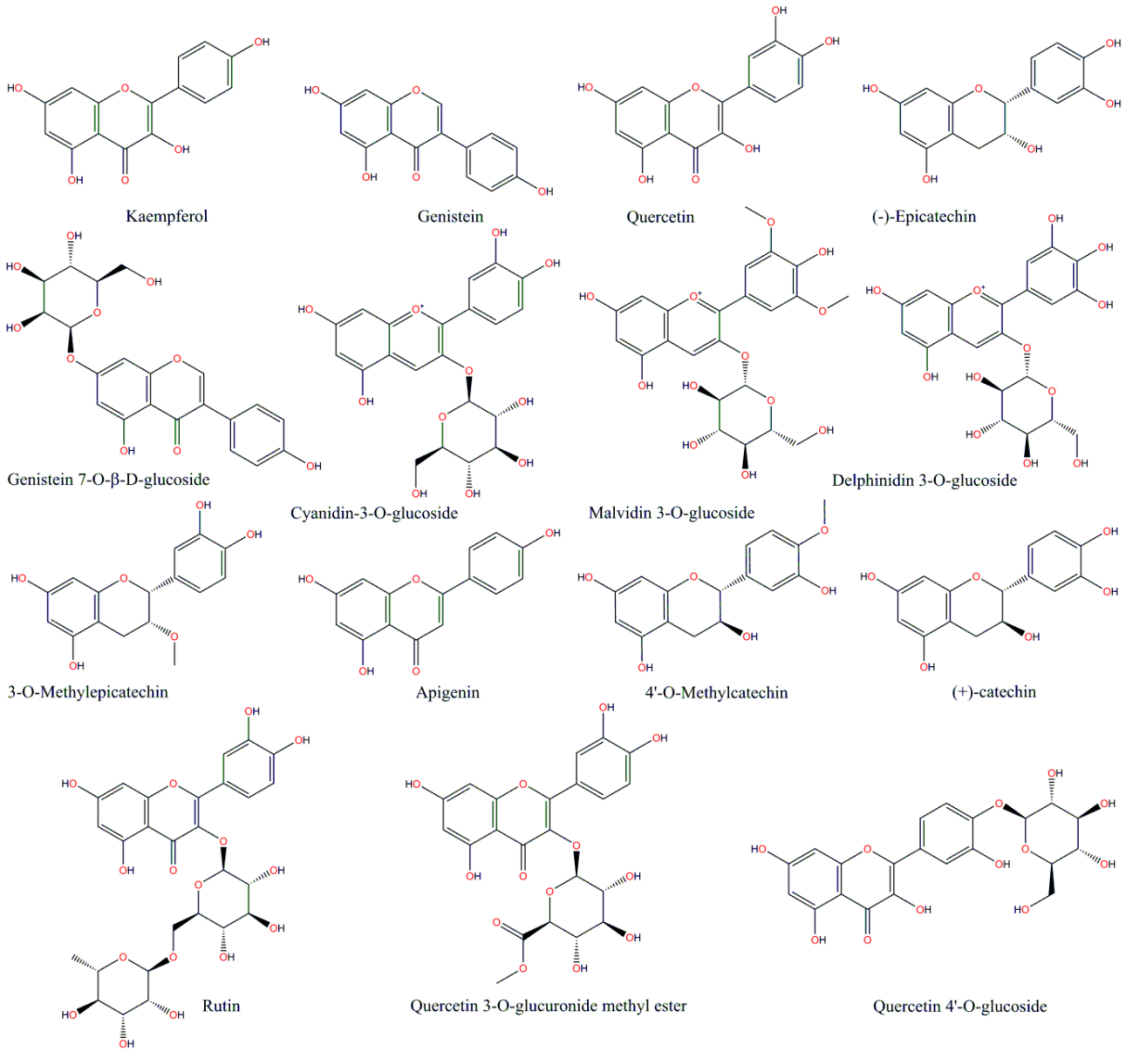

Fig.1. The 2D illustration of selected flavonoids for docking and quantum analyses.

2.2. RECEPTOR PREPARATION AND MOLECULAR DOCKING

The 3D crystallographic structures of Cdk5-p25 (PDB ID: 3O0G (Ahn *et al.*, 2005)), GSK3β (PDB ID: 1J1C (Aoki *et al.*, 2004)) and CK1δ (PDB ID: 3UYT (Long *et al.*, 2012)) as target kinases were taken up from PDB database (https://www.rcsb.org/). The importance of these receptors in the pathogenesis of AD has been formerly reported (Adler *et al.*, 2019, Llorens-Marítin *et al.*, 2014, Wilkaniec *et al.*, 2016, Wilkaniec *et al.*, 2018). First, water molecules and other heteroatoms were removed from the structure of obtained receptors. MODELLER version 9.22 software (https://salilab.org/modeller/) was applied to correct the conformation of some missing residues. Next, 10 ns molecular dynamic simulation using Gromacs version 5.0.1 tool (www.gromacs.org) was recruited to prepare the initial structure of amended receptors.

Molecular blind docking using AutoDock 4.2.6 (Trott and Olson, 2010) was carried out, and the missing hydrogens and Gasteiger/Kollman charges were assigned to the prepared system during performing docking analysis. The map of standard grid boxes for molecular docking was generated using AutoGrid 4.2.6 in which the size of grid box was 50 × 50 × 50 Å (x, y, and z) points and grid-point spacing of 0.375 Å, respectively. The number of independent docking runs performed for each docking simulation was set to 200 with

25,000,000 energy evaluations for each run. The Lamarckian genetic algorithm was also used for performing energy minimization and optimization during the time of molecular docking simulation. GaussView 3.0 tool (Frisch *et al.,* 2004) was applied to prepare graphical illustration of Gaussian outputs.

2.3. QUANTUM ANALYSES

First, TAO package (Tao and Schlegel, 2010) was recruited to isolate a sphere with 5Å radius around each studied active site to determine the electronic structures of ligand-active site complexes. All included residues in this sphere were evaluated for their electronic structures. Protoss web-server (Bietz *et al.,* 2014) was applied to protonate the isolated spheres, and Gaussian 03 (Frisch *et al.,* 2004) was used for further single point calculations at two methods including *HF/6-31G(d)* and *B3LYP/6-31G(d)*. The SCPA method was also employed to validate the contribution of molecular orbitals in each residue and/or ligand substructures using Multiwfn software (Lu and Chen, 2012). In this study, the compounds comprised both types of pure and conjugated flavonoids (methylated and glycosidic forms). Three fragments including sugar moiety, two rings of flavonoids backbone (including A and C rings) and B-ring alone were chosen as input segments for quantum chemical analysis.

3. RESULTS

3.1. INTERACTION WITH GSK3β

According to docking simulation results for GSK3β enzyme, all ligands were able to interact with the active site of this receptor by possessing binding energies ranging from -7.1 to -10.42 kcal·mol^{-1}. In comparison to crystallographic data of GSK3β active site (Aoki *et al.,* 2004), rutin, cyanidin-3-O-glucoside, malvidin-3-O-glucoside and quercetin-4'-O-glucoside posed nearby its catalytic and binding residues (***Fig. 2***). The calculated binding energies for these compounds were -10.42, -9.76, -9.30 and -9.17 kcal·mol^{-1}, respectively. Indeed, other compounds were also interacted with this active site by gaining lower docking binding energies. Accordingly**,** two oxygen atoms from OH7 of rutin A-ring and its sugar moiety (rutinose disaccharide) have formed hydrogen bonds with NE and NHE2 atoms of Arg^{141}. Another oxygen atom from OH group of 4' position of rutin B-ring also interacted with the OH of Tyr^{134} to create a hydrogen bond. The O atom of Pro^{136} also interacted with the OH group of 4'-position of rutin B-ring. Similarly, the backbone O atom of Glu^{137} interacted with the hydroxyl group of 3'-position of rutin B-ring. The NZ of Lys^{85} formed a hydrogen bond with the backbone OH group of 5-position of rutin A-ring.

The backbone N atom of Asp^{200} created a hydrogen bond with another oxygen atom of rutin. Oxygen atoms of rutin sugar were also interacted with the metal group (*i.e.* magnesium). The O atom of Gln^{185} also interacted with the OH4" group of rutin sugar moiety through H-donor interaction. The oxygen atom of rutinose moiety also formed a hydrogen bond with the NZ atom of Lys^{183}. The average distance of H-bonds between rutin and GSK3β was ~3.1Å, followed by residue-ligand atom binding energy equals to -1.8 kcal·mol^{-1}. Similar interactions were also observed for cyanidin-3-O-glucoside. The backbone N atom of Asn^{64} formed a hydrogen bond with the sugar OH group of cyaniding-3-O-glucoside. Also, the O atom of this residue interacted with the sugar unit of cyanidin-3-O-glucoside. The N atom of Asp^{200} constructed a hydrogen bond with OH7 group of A-ring of this compound. Also, Tyr^{134}, Arg^{141}, and Cys^{199} were other residues in this active site that actively participated in the interaction of this cavity with its docked ligand.

The anthocyanin malvidin-3-O-glucoside was another top docked compound that was located in the active site of GSK3β. As depicted in ***Fig. 2***, this anthocyanin interacted with

active site residues to form powerful hydrogen bonds. In addition to the above-mentioned metabolites, quercetin-4'-O-glucoside formed hydrogen bonds with GSK3β residues including Lys^{85}, Asp^{181}, Lys^{183}, Asp^{200}, Ser^{203}, and Ser^{219}, respectively. The average distance of created H-bonds per residue for quercetin-4'-O-glucoside/GSK3β active site residues was ~2.90 Å followed by residue-atom interaction energy equals to -1.5 kcal·mol^{-1}.

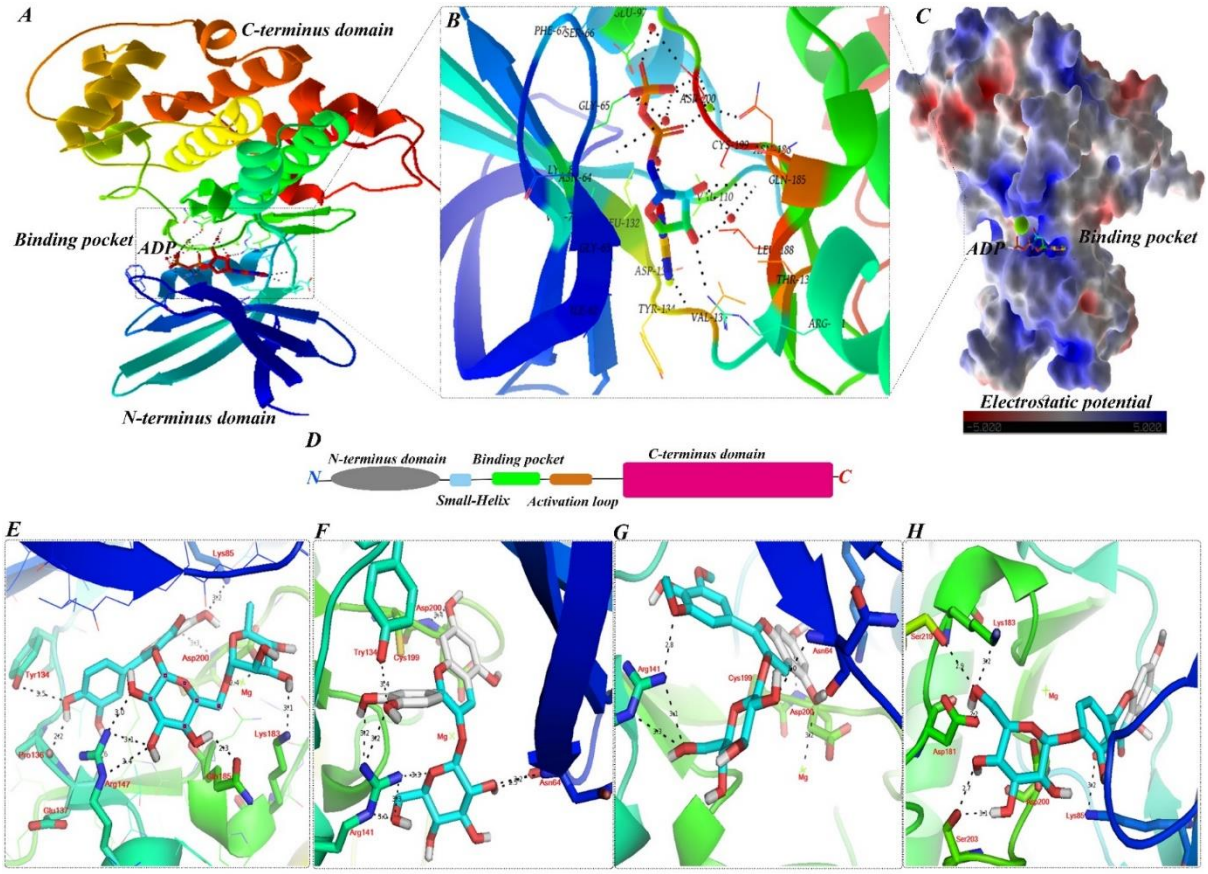

Fig. 2. Docking results for GSK3β. The structure of GSK3β (*PDB id: 1J1C(Aoki et al., 2004)*) in complex with ADP molecule (***A***), its active site binding and catalytic residues (***B***) and Poisson-Boltzmann surface electrostatic potential (***C***) are shown in this illustration. In panel (***C***) red color represents negative surface charge, blue shows positive charges and neutral regions is shown in white color. As depicted in panel (***D***), protein kinases have unique structural features including *N*- and *C*-terminus domains, small helix, binding pocket and activation loop in which possessed highly conserved catalytic residues (Aoki *et al.*, 2004, Chico *et al.*, 2009). Biochemically, conformational changes of N or C terminus domains would determine the activity protein kinases (Kokubo *et al.*, 2013). The binding mode of (***E***) rutin; (***F***) cyanidin-3-O-glucoside; (***G***) malvidin-3-O-glucoside and (***H***) quercetin-4'-O-glucoside are also shown in the bottom of the illustration. In addition to the formation of H-bonds, compounds ***E*** and ***G*** were also interacted with Mg ion in this active site.

3.2. INTERACTION WITH CDK5-p25

The docking outcomes for Cdk5-p25 enzyme and selected flavonoids showed that these compounds have also potentially interacted with the active site residues of this enzyme. Rutin, delphinidin-3-O-glucoside, malvidin-3-O-glucoside and quercetin-4'-O-glucoside exhibited potent docking binding energies. The binding energy values of these top

compounds were -10.0, -9.89, -8.10 and -7.02 kcal·mol^{-1}, followed by the average H-bond distance of ~2.1 Å, and residue-atom interaction energy equals to -2 kcal·mol^{-1}, respectively.

Binding mode analysis of top docked compounds showed that these ligands formed both H-donor and H-acceptor interactions within this active site. Crystallographic data indicated that several residues including Leu^{10}, Glu^{12}, Val^{18}, Phe^{80}, Glu^{81}, Phe^{82}, Cys^{83}, Asp^{84}, Glu^{85}, Asp^{86}, Leu^{133} and Asn^{144} (or Asp^{144}) were participated in the formation of H-bonds or other non-covalent interactions with the backbone of small molecule inhibitors (Ahn *et al.*, 2005). The docking results for studied compounds showed that docked flavonoids could easily enter the cavity Cdk5-p25 active site and formed several H-bonds with its catalytic and binding amino acids.

Interestingly, the OH7 group of quercetin-4'-O-glucoside constructed a non-covalent H-π interaction with benzyl side chain of Phe^{80} residue. The formed hydrogen bonds between the docked compounds and Cdk5-p25 active site residues indicated that these ligands have the potential to facilitate the disruption of its normal biological activity (Ahn *et al.*, 2005). In this regard, recent enzymatic investigation indicated that some flavonoid-based inhibitors of Cdk5-p25 tightly interacted with its catalytic residues and possessed IC_{50} value equal to 4.81µM, respectively (Shrestha *et al.*, 2013).

Studies showed that the entrance of ligands into the active site cavity is simultaneously associated with the possible changes in active site cavity shape and function (Ghazvini *et al.*, 2018, Rasouli *et al.*, 2017). The essence of active site residues (acidic, hydrophobic, *etc*.) and chemical structure of inhibitors might function as two determinant factors to affect the possible interaction of ligands and receptors (Rasouli *et al.*, 2018). In comparison to docking outcomes of GSK3β, the calculated docking energies were not greater, and the number of formed H-bonds were also decreased (***Fig. 3***). This may indicate that the studied metabolites might have a dose-dependent inhibitory profile for this enzyme *in vitro* or *in vivo.*

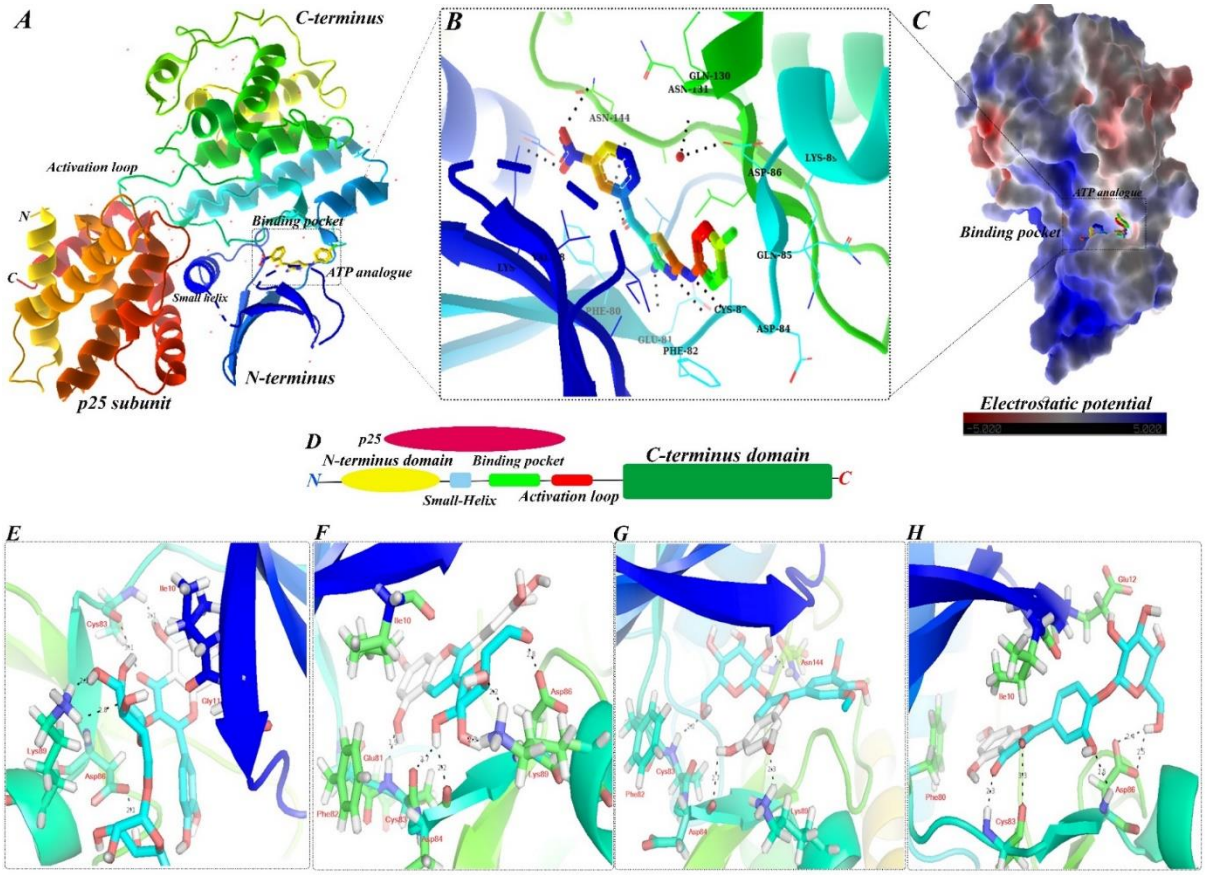

Fig. 3. Docking results for Cdk5-p25 (*PDB id*: 3O0G). Panels (***A***) the conformational arrangement of CdK5-p25 in complex with its inhibitor; (***B***) illustration of its critical active site amino acids; (***C***) electrostatic surface potential and (***D***) simplified view of CdK5-p25 structure. P25 subunit regulates the activation of this enzyme and without this fragment Cdk5-p25 cannot handle its molecular functions (Tarricone *et al.*, 2001). Top docked ligands including (***E***) rutin; (***F***) delphinidin-3-O-glucoside; (***G***) malvidin-3-O-glucoside and (***H***) quercetin-4'-O-glucoside are shown the bottom sections of this illustration.

3.3. INTERACTION WITH CK1δ

The detailed information on most important residues of this active site formerly discussed (Long *et al.*, 2012). Docking results for CK1δ enzyme displayed that four metabolites including cyaniding-3-O-glucoside, 4'-methylcatechin, 3-O-methylepicatechin and delphinidin-3-O-glucoside were tightly bound to the active site cavity of CK1δ through forming covalent and non-covalent bridges (***Fig. 4***). The calculated binding energies for top docked compounds were -8.11, -8.0, -7.30 and -7.0 kcal·mol^{-1}, respectively. Both types of H-acceptor and H-donor interactions were observed between ligands atoms and active site amino acids. Additionally, CD1 atom of Ile^{23} formed a non-covalent H-π interaction with A-ring of 4'-methylcatechin. The docking results demonstrate that flavonoids compounds have lower binding affinities for this receptor in comparison to GSK3β and Cdk5-p25.

The size of active site cavity, molecular weights and chemical structures of docked ligands and tendency of ligands to interact with this active may be considered as potential factors to inhibit this enzyme. However, the construction of H-bonding and other types of chemical interactions between flavonoids backbone atoms and CK1δ active site amino acids including

Ser[11], Ile[15], Ile[23], Lys[38], Leu[85], Asp[91], Tyr[65], Lys[130], Asp[132] and Asn[133] residues demonstrated that this enzyme is one the possible targets flavonoids metabolites can interact with its active site after entrance the CNS environment.

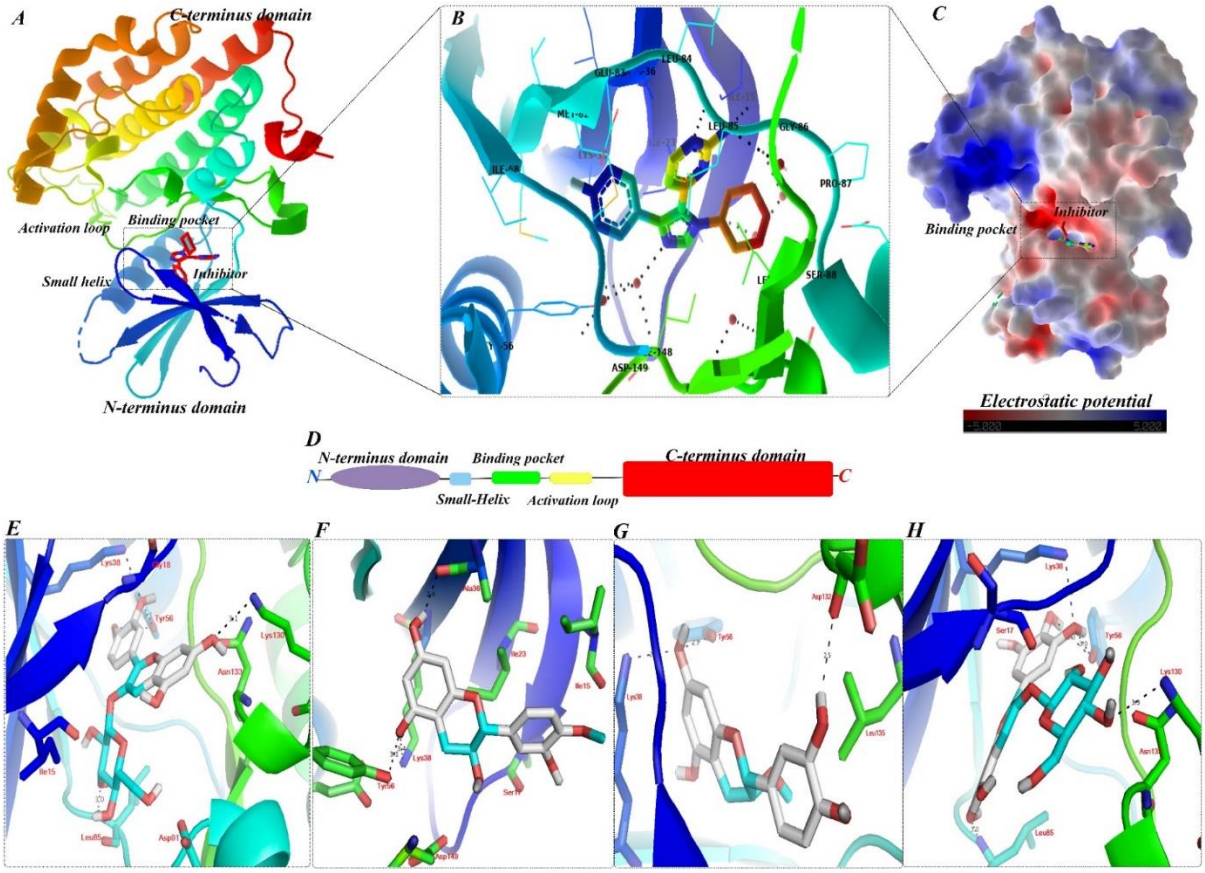

Fig. 4. Docking results for CK1δ. Sections ***A-D*** depicted structural features of CK1δ enzyme (*PDB id: 3UYT (Long et al., 2012)*). The binding mode of (***E***) cyaniding-3-O-glucoside; (***F***) 4'-methylcatechin; (***G***) 3-O-methylepicatechin and (***H***) delphinidin-3-O-glucoside are shown in the bottom of this illustration.

Apigenin, kaempferol, genistein and quercetin accordingly displayed similar docking binding affinity energies against kinases. With binding energies of -6.0 kcal·mol^{-1}, followed by the average H-bond distance of 2.1 Å, their docking almost showed similar affinities during docking simulations. Additionally, (-)-epicatechin and (+)-catechin showed acceptable binding energy values for GSK3β (-8.01 and -7.30 kcal·mol^{-1}) and Cdk5-p25 (-6.5 and -5.0 kcal·mol^{-1}) active sties. Quercetin 3-O-glucuronide methyl ester and genistein 7-O-β glucoside also showed similar binding energies for GSK3β (-5.5 kcal·mol^{-1}) and CK1δ (-5.0 kcal·mol^{-1}) receptors. In summary, all compounds could interact with catalytic residues of target active sites, and it seems the variability of binding energies is related to chemical structure of these compounds and tendency of active site residues to participate in the occurred interactions.

In the following sections, we will show how distribution of molecular orbitals among ligands backbone fragments or active site residues might provide valuable information on the effectiveness of ligand-receptor interactions.

3.4. QUANTUM RESULTS

According to quantum chemical results, the considered fragments including A/C scaffold, B-ring and sugar moiety unraveled different functions in the structure of studied ligands. Additionally, active site residues also shared similar behaviors, showing a specific pattern of molecular orbital distribution in their structure. By considering the isolated 5Å radius spheres from each active site, the quantum results suggested that each residue has a specific contribution in the distribution of molecular orbital levels. In both methods, considerable changes of MOs have been observed for ligand-receptor complexes and free structures (***Suppl. File S1- Table 1 to 5-1***). Indeed, based upon the chemical backbone of active site residues variable contributions have been found for studied active site amino acids (***Suppl. Fig. A1***).

This finding significantly supports our understanding of the primary roles of LUMO and higher LUMO orbitals to address the participation of active site residues to interact with ligands and/or their substructures in the ligand-receptor complex. Fukui *et al*. (Fukui *et al.*, 1954, Fukui *et al.*, 1952) applied the FMO theory to predict the process of chemical reactions, and this approach has clearly changed chemical views to understand the basic essence of chemical reactions and associated interactions. Hoffmann and Woodward (Hoffmann and Woodward, 1965, Hoffmann and Woodward, 1968) developed the FMO theory to highlight the critical roles of orbital symmetries in this concept. To overcome limitations with the gap between HOMO and LUMO orbitals, researchers have applied the FERMO concept (da Silva *et al.*, 2006) to predict the chemical behavior of ligands precisely.

According to FERMO theory, the chemical action of ligands in the first encounter with the receptors was determined by the shape and distribution of MOs (Silva *et al.*, 2006). Therefore, there is a possibility for ligands to possess various FERMOs to interact with the receptor active site residues specifically. As a result, a FERMO can be a HOMO orbital or other frontier orbitals (Silva *et al.*, 2006). Furthermore, the behavior of active site residues (both catalytic and binding amino acids) is a way to interpret the concept of hardness and softness, thus, the involvement of different substructures will be considered as FERMO (donor) and LUMO or higher LUMO (acceptor). Similarly, Klopman recruited a new concept called the charge and/or frontier-controlled reactions (Klopman, 1968) by considering Pearson's HSAB concept (Pearson, 1963).

Accordingly, when *Egap* of FERMO (donor) and/or higher LUMO (acceptor) possesses a higher value; consequently, the reaction is charge controlled, and such condition describes the nature of hard-hard interactions (Pearson, 1963). Similarly, smaller amounts of this value predicted the frontier-controlled reaction and soft-soft interaction (Klopman, 1968, Pearson, 1963). Considerably, much excitement generated from KS and HF molecular orbitals that showed similar behaviors to explain FERMO and LUMO in different datasets (some different behaviors for these molecular orbitals also reported) (da Silva *et al.*, 2006, Maksić and Vianello, 2006, Silva *et al.*, 2006).

The main reason for this phenomenon might be related to the intrinsic weakness of the KS molecular orbitals which will affect the effectiveness of outcomes. Knowing this issue helps us to apply both HF and DFT (B3LYP) methods to calculate molecular orbitals. Because of the higher accuracy of HF molecular orbitals compared to practical results (Ramalho *et al.*, 2003), both HF and B3LYP results were also used to interpret quantum chemical outcomes. Meanwhile, the role of each active site residue in the expected interactions was also surveyed to find their best contribution with molecular orbitals. As depicted in ***Fig.5***, the studied active site residues displayed a variable distribution in behavior, shape and rate of ΔE_{gap} HOMO and

LUMO orbitals based on distribution's map of molecular orbitals in HOMO and LUMO levels.

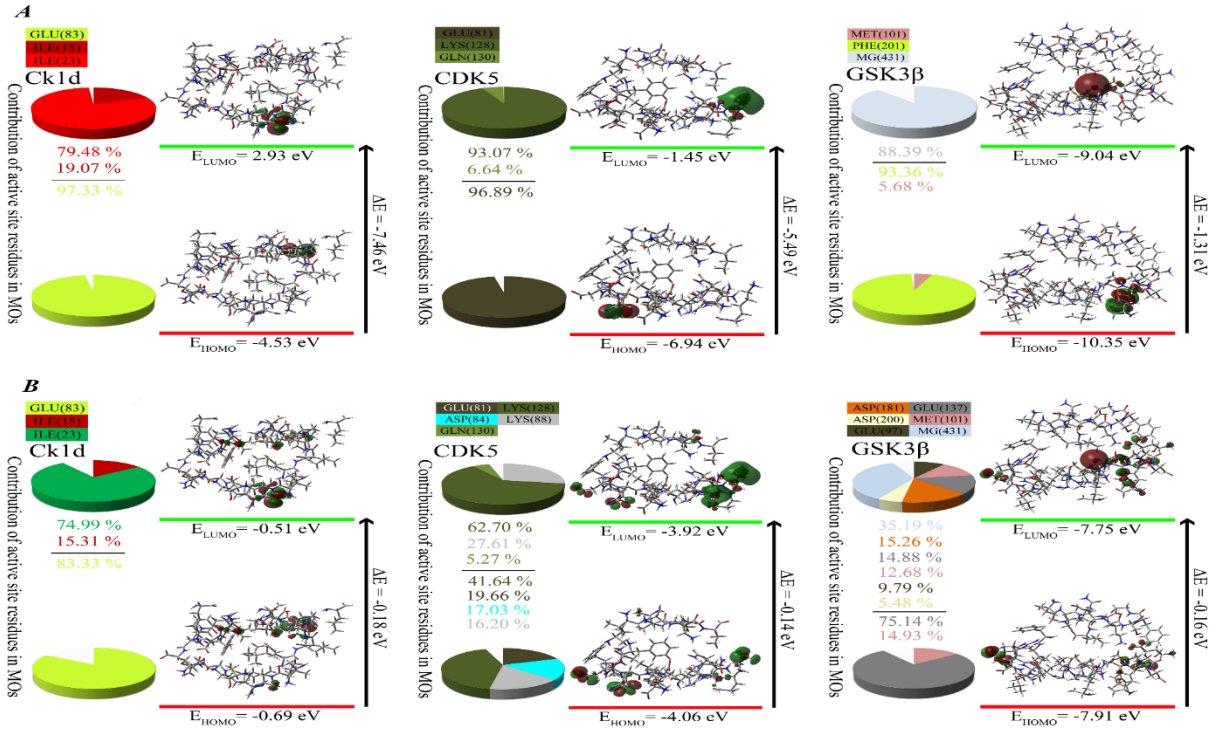

Fig.5. The participation of active site residues in MOs at (***A***) HF and (***B***) B3LYP methods. As shown, target residues showed a considerable role in the distribution of molecular frontier orbitals. These results indicate that simultaneous application of HF/B3LYP methods is a reliable approach to generate high-quality quantum chemical outcomes. In both methods, Mg ion of GSK3β active site contributed to the sharing of MOs within the active site. Intriguingly, this ion formed strong H-bonds with some of docked flavonoids and these calculations proved its critical role herein. The variability of active site residues contributions speculated that the functionality of each active site is depending on the participation of these residues to interact with nearby ligands. Indeed, numerical values of ΔE_{gaps} for residual contribution to the dissemination of MOs are different at each method.

According to the calculated values of ΔE_{gap} HOMO and LUMO orbitals (at both methods), the studied fragments demonstrated a considerable variation in the first encounter of the ligand-receptor complex for sharing their role in the distribution of MOs (***Fig.6***). The distribution of MOs in the structure of each ligand depends on the substituted chemical groups attached to the ligand skeleton, playing a pivotal role in the interaction of ligands with favorable receptor amino acids in coincidence with the shared molecular orbitals of active site residues.

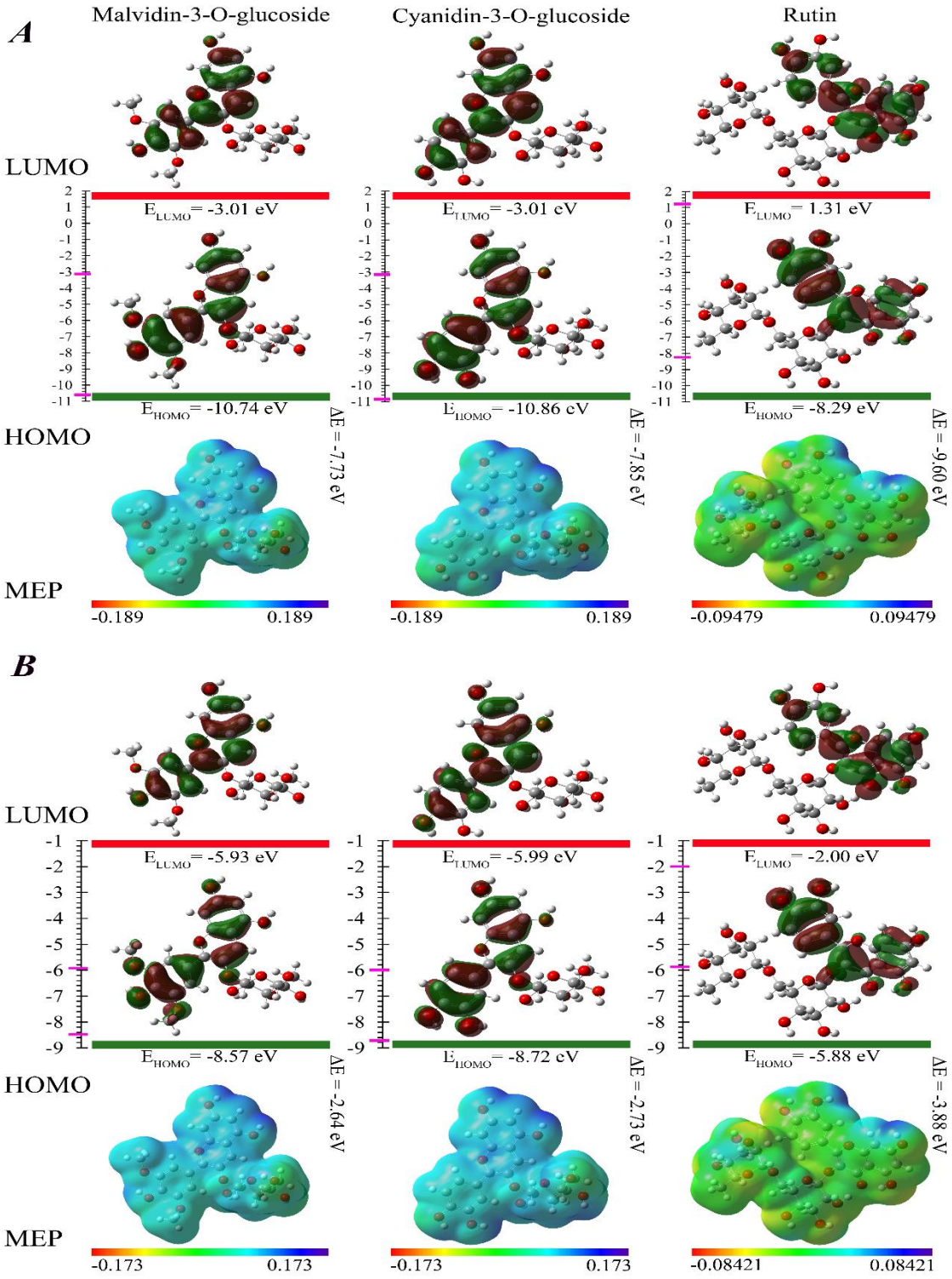

Fig.6. The calculated HOMO/LUMO and ΔE_{gaps} for studied ligands at (***A***) HF and (***B***) B3LYP methods. As shown, the chemical backbones of selected compounds display significant variation in the distribution of frontier molecular orbitals and MEPs. This means that the chemical nature of flavonoids backbone and its attached moieties have an impact on the distribution of molecular orbitals. MEP surfaces show that dispersion of positive/negative charges of ligand structures was partially different at recruited quantum methods. In each MEP surface, red color represented the distribution of negative charges where the blue functioned opposite. Indeed, the red zone is favorable for electrophilic attacks and vice versa.

Indeed, the results demonstrate that the chemical structure of rutin is harder than cyanidin-3-O-glucoside, and malvidin-3-O-glucoside (***Suppl. File S2- Table 1***). Additionally, these calculations to analyze of the electronic configuration of ligand-protein complex (in a sphere via 5Å radius obtained from docking outputs), optimized free ligand and protein alone were displayed that the ligand-protein complex is harder than other free structures, and the hardness confirmed the deterrence (or absorption) of ligand against studied active sites (***Suppl. File S2- Table 1***). According to the HSAB concept, both "hard and soft" terms are applying for determining orbitals overlap to particular chemical cases, as addressed in the above-mentioned supplementary files. The application of the HSAB concept for prediction of inhibitor-target interactions is formerly documented to predict potential electrophilic/nucleophilic attacks within target active sites (LoPachin *et al.*, 2012). In this regard, the studied compounds displayed different chemical hardness/softness values by which such features may determine the effectiveness of their backbone to correctly interact with a set of catalytic/binding residues in the active site of target enzymes.

The results of the electronic structures also revealed that the sugar moiety displayed a weak role in the contribution of molecular orbitals (around HOMO and LUMO), thereby, it functions as a donor group in the structure of ligands (***Fig.7***). For this fragment, the highest contribution in *HOMO -3* (for C3G and M3G) and *HOMO -4* (for rutin) orbitals were observed while it showed the lowest participation in *empty LUMO* and *LUMO -6* orbitals. However, this data speculates that the sugar group may participate in the distribution of molecular orbitals of protein (LUMO and *higher LUMO*). Indeed, the data of LUMO and higher LUMO orbitals has suggested that the participation of active site residues in the LUMO orbitals determined the type of interaction (*i.e.*, soft-soft or hard-hard) and the kind of control (*i.e.*, frontier and charge controlled). In summary, the computational results of quantum chemical evaluations demonstrate the importance of molecular orbitals distribution in the structure of ligands, which affected by the presence of sugar moiety, played a significant function in the interaction of ligands or their substructures with active site residues.

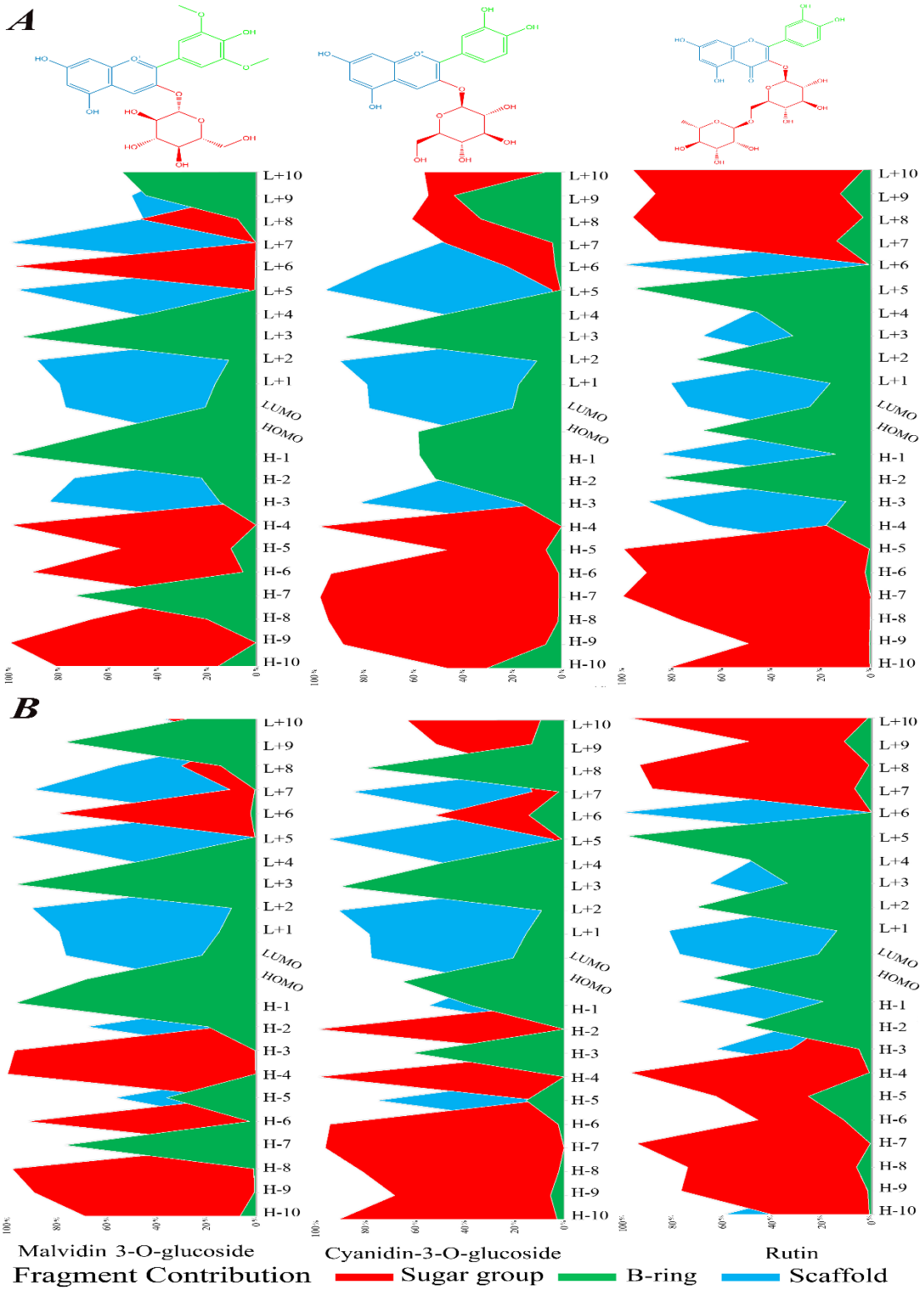

Fig.7. The role of studied fragments in the distribution of MOs at (***A***) HF and (***B***) B3LYP methods. The B-ring fragment showed maximum participation in the distribution of HOMO orbital and acts as a donor group. Also, the A/C rings scaffold fragment (blue color) displayed higher participation in LUMO orbital and functions as an acceptor group.

3.5. STRUCTURE-ACTIVITY RELATIONSHIPS

As detailed in ***Fig.8***, the sugar fragment has a high chance to interact with a variety of peripheral, inner/outer residues of studied active sites in comparison to B-ring and A/C scaffold fragments. Applying occupied (HOMO and *lower HOMO*) and unoccupied (LUMO and *higher LUMO*) orbitals to investigate the role of ligand substructures in the distribution of molecular orbitals revealed that the sugar moiety of the studied ligands has a highest distribution in the molecular orbitals (HOMO to *HOMO -10*); consequently, this functional chemical group is able to transfer electron to the side of unoccupied orbitals in protein active site. Similarly, the scaffold fragment shared maximum involvement in the distribution of unoccupied orbitals, chemically could receive electron from protein.

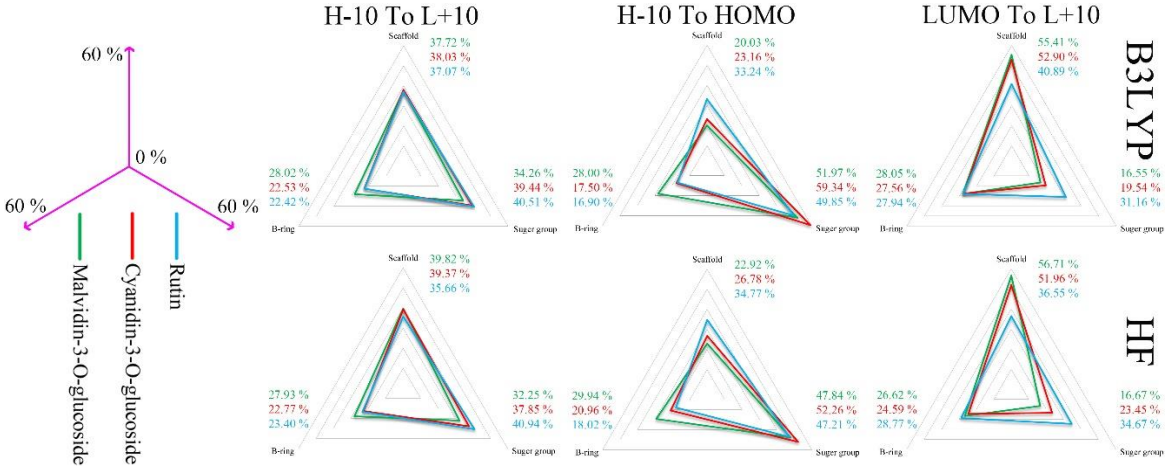

Fig.8. The total percentage of MO distributions of studied fragments including B-ring, sugar moiety and A/C rings of backbone scaffold. The numerical values indicated the portion of each moiety in the distribution of MOs. The maximum percentage of calculated MOs for each compound shown in green, blue and red colors.

4. DISCUSSION

Various reports confirmed the neuroprotective effect of flavonoids. Rivera *et al*. (Rivera *et al.*, 2004) reported that the neuroprotective effect of flavonoids is associated with their chemical structures (Rivera *et al.*, 2004). Das *et al*. (Das *et al.*, 2017) computationally investigated the anti-Alzheimer activity of several flavonoids against AChE enzyme and nominated some of them as possible targets for further studies. Other studies (Trebaticka and Ďuračková, 2015) also reviewed the health benefits of polyphenols against metal disorders and suggested that these compounds are valuable natural medications to alleviate the oxidative stress of neurodegenerative diseases. Similarly, Ayaz *et al*. (Ayaz *et al.*, 2019) addressed the effectiveness of flavonoids as potent biomolecules in the hallmark of aging and other neurological disorders prevention.

In the wake of docking simulation drawbacks, researches have been documented that most of docking limitations are owing to the lack of accurate scoring function to highlight the essence of protein-ligand interactions at atomistic levels (Kokubo *et al.*, 2013). Available algorithms, despite their popularity, may not wholly meet up the requirements for showing how ligands substructures, functional groups or central backbone atoms might change pouring of electron flow into protein active site cavity (Raha and Merz, 2004, Rasouli *et al.*, 2017).

The information of interacted ligand-protein complex helps researchers to understand physical behavior, which means visible interaction of ligand-protein complex, of ligands after gluing to their target residues. Accordingly, the functional roles of active site associated chemical additions such as water molecules or ions have widely been ruled out by docking simulation tools because there has rarely been a proper scoring model to explicit the behavior of these chemical molecules during ligand-protein interlinkage (Kokubo *et al.*, 2013).

For instance, the results demonstrated that Mg ion of GSK3β active site participated in the distribution of MOs at both methods, formed H-bonds with the side chain of docked ligands while docking outcomes only highlighted its role for interaction with two ligands. Therefore, docking scoring problems loom large when virtual screening pipelines applied such scoring functions or predictive models to filter and screen top hits of large drug-like libraries for further pharmaceutical application (Korb *et al.*, 2012, Raha and Merz, 2004).

Previously, studies revealed that coupling quantum-based data with docking results could provide new structural insight into binding mode of phenolic compounds and carbohydrate digestive enzymes (Rasouli *et al.*, 2017). Discrimination of top docked ligands by considering both docking binding energies and distribution of FMOs can help researchers to interpret docking results accurately. Other studies (Wasukan *et al.*, 2019) reported that combining QM calculations and docking results provided deeper understanding of silver nanoparticle-mediated cytochrome P450 inhibition. More interestingly, Lukac *et al*. (Lukac *et al.*, 2019) have shown that the combination of QM outcomes with calculated or predicted Log*P* values was a reliable approach to improve the accuracy of protein-ligand binding energies. Very recently, researchers (Cavasotto *et al.*, 2018) reviewed the importance and functionality of QM-based calculations in protein-ligand docking and reported that these methods could provide invaluable information about the chemical basis of protein-ligand interactions at atomistic levels.

Although QM analyses were applied to improve of protein-ligand binding energies calculations, two problems including *cost of computation time/hardware for each step of QM analysis* (Raha and Merz, 2004) and *general uncertainty of docking scoring functions* limited large-scale utilizations of these methods to screen millions of chemical compounds (Cavasotto *et al.*, 2018). Indeed, the current literature does not investigate the simultaneous distribution of quantum MOs throughout ligand backbone and active site cavity at different levels, instead inserting greater emphasis on small molecule inhibitors after interaction with protein amino acids. However, this data was displayed that a possible change in the numerical values of quantum MOs is associated with both substitution of ligand backbone functional groups and specificity of active site amino acids side chains. Additionally, at two studied QM methods, ligands and the nearby amino acids showed their contribution to share favorable MOs by which the portion of most important studied fragments judged. At all, quantum chemical analysis is a trustworthy approach to study a variety of chemical reactions, chemical bonds (Ghafari and Gholipour, 2015, Jooneghani and Gholipour, 2019, Solimannejad and Ghafari, 2013) and other features related to both ligands and receptors to provide new structural insights into molecular interaction of inhibitors and potential active sites.

Findings suggest that the chemical modification of flavonoids skeleton determined the effectiveness of these ligands to interact with target amino acids. The structural variability of flavonoids is thought to be a valuable potency to alter the normal function of their targets by providing dose-dependent inhibitory properties (Rasouli *et al.*, 2017, Rasouli *et al.*, 2018, Rasouli *et al.*, 2018). It seems obvious that the variation and/or the alternation of attached –OH groups to flavonoid backbones (at B/C-rings and C2=C3 positions) could change their toxicity effect against the aggregation of Aβ (Lee *et al.*, 2016). Also, other researchers have been documented that the biological activity of flavonoids is directly associated with the variability of their chemical structure (Rasouli *et al.*, 2018, Xiao, 2017). The B-ring of rutin,

C3G and M3G have different numbers of –OH groups and C3G and M3G have the same sugar group at the C2=C3 position, while rutin has two sugar units in this position. Thus, these chemical substitutions will effectively modify the tendency of these ligands to bind to active site amino acids.

Other investigations also suggest that the acetylation of flavonoids backbone at different positions may change the biological functionality of these metabolites (Chebil *et al.*, 2007). Interestingly, the antioxidant activity of flavonoids is directly linked to the number of attached –OH groups to their central skeleton and without these chemical moieties are not able to affect cellular signaling pathways (Chen *et al.*, 2017). The growing body of evidence also speculates that antioxidant and the inhibitory profile of some flavonoid compounds against protein kinase Cδ receptor are correlated with the number of B-ring hydroxyl groups (Kongpichitchoke *et al.*, 2015). Generally, different factors such as hydroxylation, methylation, C2=C3 double bond, the –OH linked to the C3 position, glycosylation, acetylation and the number of –OH groups are caused various modifications in flavonoids backbones and changed their biological behavior within the cell (Xiao, 2017).

As a biological standpoint, targeting τ protein kinases using small molecule inhibitors should consider a list of pros and cons to overcome the available challenges with current strategies to design anti-Alzheimer drugs (Chico *et al.*, 2009, Martin *et al.*, 2013). An important point should be addressed is that bioavailability and pharmacokinetics of drug-like compounds will define their functional inhibitory properties because most of these compounds are not reached CNS *in vivo* (Trebaticka and Ďuračková, 2015). More importantly, computational analyses only displayed how do active site residues selectively (or randomly) interact with a variety of chemical substances (Ghazvini *et al.*, 2018, Rasouli *et al.*, 2017), and these outcomes are effective whenever clinical intervention studies approve the quality of natural products for alleviating human diseases (Yella *et al.*, 2018).

Recommending natural products as promising medications for the treatment of human diseases is not a primitive strategy and this is mainly ascribed to considerable numbers of biological factors (*e.g.* genetic factors, body metabolism, biological barriers, plasma proteins, target resistance, active site mutations, reactivity of structural moieties, instability, low solubility, immunological responses, interaction with other cellular targets, violation from filtering systems such as *Lipinski Rule of Five*, *etc*.) may simply change the expected biological properties of natural ligands (Amirkia and Heinrich, 2014, Press *et al.*, 2019). Specification of flavonoids backbones to interact with a variety of protein kinases by improving flavonoids selectivity and affinity, bioavailability, metabolism and designing future smart flavonoids using coupling advanced chemical computations and *in vivo* studies will decrease the number of clinical failures and improve the applicability of introduced compounds for AD prevention. By and large, greater focuses should be inserted on the transportability through physical barriers and metabolism of newly designed drugs within the body for long-term clinical success against AD (Chico *et al.*, 2009).

The outcomes represented that in addition to docking physical interactions, other chemical influential forces such as distribution of MOs between active site residues and chemical moieties of ligands (or their substructures) determine the "*type of interaction*" between ligands and receptors. As shown, molecular orbitals are quintessential parts of each chemical interaction, and considering their functional roles for expansion drug-like scaffolds may lower the total yield of virtual screening outcomes but produce promising backbones for further studies. Developing MOs descriptors using huge chemical libraries is highly recommended and such outputs will provide valuable treasures of quantum data for interpretation and filtering of virtual screening results. The simultaneous application of docking and QCAs coupled with other high-quality *in silico* methods such as molecular dynamic simulation, machine learning, data-mining, and QSAR validations might offer an

unprecedented opportunity to highlight all possible anti-Alzheimer drug-like candidates by applying heterogeneous datasets generated from these methods.

5. CONCLUDING REMARKS

The results herein showed that flavonoids metabolites might interact with brain protein kinases but the variation of their backbone would lead to an increase or decrease in their binding energies. The outcomes were also shown that some flavonoid metabolites could interact with more than two kinases; therefore, these ingredients might have several targets within the brain. Interestingly, coupling chemical quantum analysis with docking results highlighted the functional roles of molecular orbital distribution between ligands and receptors in the determinations of possible interactions among studied complexes. The overall finding from this research may be widely applicable for drug design experts in the extraction of molecular mode of action of flavonoids using coupling advanced computational methods with available methods. Whether this premise is true or not, further development of this finding to approve its efficacy is strongly acknowledged.

CONFLICT OF INTEREST

The authors declare no competing financial interest.

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