The Journal of Membrane Biology
https://doi.org/10.1007/s00232-019-00105-1

The Interaction of Flavonols with Membrane Components: Potential Effect on Antioxidant Activity

Sarmistha Saha[1] · Emiliano Panieri[2] · Sibel Suzen[3] · Luciano Saso[2]

Received: 20 October 2019 / Accepted: 14 December 2019

Abstract

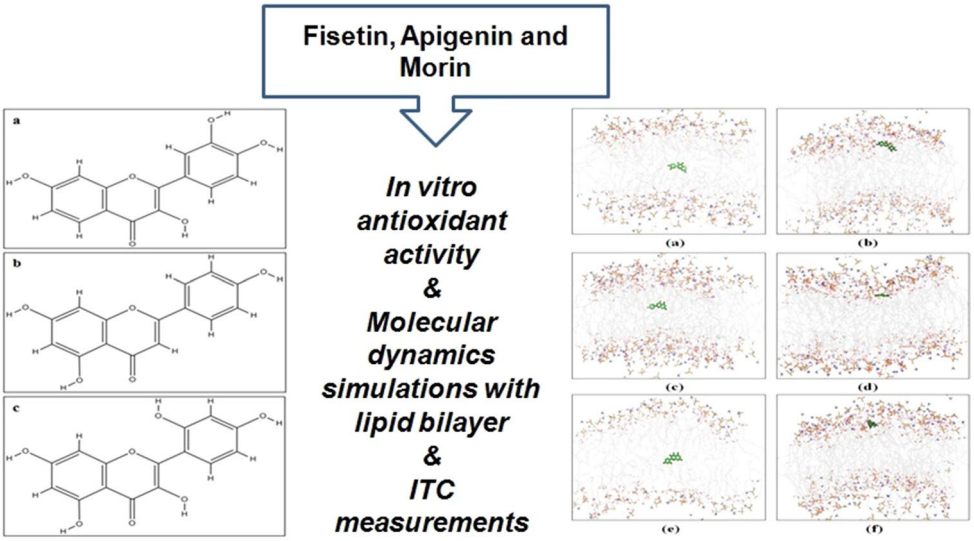
Flavonols are the most widely distributed class of dietary flavonoids with a wide range of pharmacological properties due to their potent lipid peroxidation inhibition activity. The permeability and orientation of these compounds in lipid bilayers can provide an understanding of their antioxidant and lipid-peroxidation inhibition activity based on their structures at the molecular level. For this purpose, we studied antioxidant activity and atomic-scale molecular dynamics simulations of 3-hydroxyflavone (fisetin), 5-hydroxyflavone (apigenin) and 3,5-hydroxyflavone (morin) in palmitoyloleylphosphatidylcholine (POPC) membrane models with 0 mol% and 40 mol% cholesterol concentration. In pure POPC bilayer with 0 mol% cholesterol concentration, the flavonols penetrated into bilayer with lowest free energy profiles, however, incorporation of 40% cholesterol concentration reduced the permeability of the flavonols. Higher cholesterol concentrations in the POPC lipid bilayer resulted in an increase of the bilayer thickness and corresponding decrease in the area per lipid which rationalizes the reduced partitioning of flavonols due to cholesterol. In the presence of cholesterol, the flavonols reside at the polar interfacial region of the lipid bilayer to form higher H-bonding interactions with cholesterol molecules in addition to water and lipid oxygens. Among all the selected flavonols, morin showed the highest affinity which was driven by the hydrophobic effect as also depicted by ITC (Isothermal titration calorimetry) experiments and thus, more efficient antioxidant in scavenging superoxide, nitric oxide radicals as well as lipid peroxyl radicals. Furthermore, our simulations also confirmed that the permeability of compounds is sensitive towards the cholesterol content in the membrane.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00232-019-00105-1) contains supplementary material, which is available to authorized users.

Extended author information available on the last page of the article

Published online: 02 January 2020

Graphic Abstract



Keywords Flavonols · POPC · Cholesterol · Lipid peroxidation · Molecular dynamics simulations

Introduction

The affinity of dietary flavonoids to lipid bilayers of cell membranes could be a key for their antioxidant activity towards the lipid peroxidation in several pathophysiological conditions. Dietary flavonoids are polyphenols that are widely distributed in fruits and vegetables and constitute a major portion of the human diet (Panche et al. 2016). They are well known due to their vast biological activity against oxidative stress, diabetes, inflammation, microbial diseases, and cancer (Romano et al. 2013). They have been claimed to be a key for new therapeutic strategies because of their potential antioxidant activity which has been attributed to their structural features, including hydroxyl, methoxy, nitro, methyl groups attached to the two aromatic rings (A and B) linked with a three-carbon chain, organized as an oxygenated heterocycle (ring C) (Gonçalves and Romano 2017). In this regard, flavonols constitute the most abundant class of dietary flavonoids and found virtually in all vegetables and fruits. Flavonols show a wide range of biological properties like antioxidant (D'Andrea 2015), anti-inflammatory (Aguilera et al. 2016), antiatherosclerotic (Mao et al. 2017), anticancer (Davatgaran-Taghipour et al. 2017), and antidiabetic activities (Asgar 2013).

The interplay between antioxidant systems and the production of oxidants including reactive oxygen species (ROS) and reactive nitrogen species (RNS) play an important role in many diseases, especially cancers, inflammatory diseases, Alzheimer's disease, and cardiovascular diseases (Gurer-Orhan et al. 2018). The unique ability of flavonoids to influence the lipid peroxidation in different pathological conditions implicates the correlation of their diverse functions with their interactions with biomembranes (Evans and Holian 1985). However, relatively little is known about the details of how flavonoids interact with lipid components (Sanver et al. 2016). The ability of flavonoids to inhibit lipid peroxidation is undoubtedly related to their interaction with the membrane lipid bilayers. However, despite the work done regarding pharmacological effects of flavonoids, there are still controversies regarding their penetration and positioning into the lipid bilayers. In one study, the well-known antioxidant, quercetin was found to be inserted deeply within phospholipid bilayers (Saija et al.1995). However, in the same report, another flavonoid, rutin does not appear to interact with model membranes, although it showed strong antioxidant property against linoleate oxidation (Saija et al. 1995).

Biological membranes are composed of a variety of lipids, proteins, and cholesterol. The penetration and the permeability of the compounds in the lipid bilayers are driven by the membrane composition, level of unsaturation, cholesterol content, and the properties of the permeating compound (Shinoda 2016). Cholesterol content in the plasma membrane of animal cells ranging from 20 to 40% (Neuvonen et al. 2014) is an important factor in the maintenance of the proper fluidity and rigidity of the membranes (Roìg et al. 2009).

Earlier studies showed a moderate reduction of membrane permeability for water, and ions due to cholesterol (Pandit et al. 2008). Furthermore, wet-lab experiments and

computational studies demonstrated the effect of cholesterol on the partitioning of water or molecular oxygen into lipid membranes in membranes of unsaturated lipids (Subczynski et al. 1994). In one study, structure-dependent interactions of different flavonoids with phospholipid monolayers on a mercury film electrode were performed by rapid cyclic voltammetry. The results showed that flavonoids implementing a planar configuration improved the membrane properties more than non-planar flavonoids (Sanver et al. 2016). The inhibition of lipid peroxidation and other related bioactivities of the antioxidants could be related to their ability to interact with lipid bilayers. Therefore, it is worthwhile to study and differentiate the distribution and orientation of flavonol compounds in an unsaturated lipid bilayer and the influence of cholesterol on the permeability of these compounds into biological membranes. In this context, we have selected flavonols based on hydroxyl group at position 3 (3-hydroxyflavone, fisetin), at position 5 (5-hydroxyflavone, apigenin) and with hydroxyl groups at both positions (morin).

Fisetin (3,7,3′,4′-tetrahydroxyflavone) occurs in many edible vegetables and fruits, such as apples, strawberries, grapes, and onions with antioxidant (Hanneken et al. 2006; Zhang et al. 2019), neuroprotective (Maher et al. 2006; Ahmad et al. 2017), anti-proliferative (Haddad et al. 2006; Fu et al. 2019), anticancer (Pal et al. 2013; Kashyap et al. 2018), and anti-inflammatory properties (Higa et al. 2003; Peng et al. 2018). Fisetin was found to suppress the proliferation of human melanoma cells both in vitro and in vivo by inducing apoptosis in human prostate cancer cells and through the disruption of Wnt/β-catenin signaling (Syed et al. 2011). More recent evidence also showed that fisetin can inhibit proliferation, migration, and inhibition of human mammary carcinoma cell lines by interfering with the PI3K/Akt/mTOR signaling pathway (Sun et al. 2018). Anticancer property of fisetin is also mediated by cell cycle arrest, caspase-dependent apoptosis, and modulation of the MAPK and NF-κB pathways (Youns and Abdel 2017).

Similarly, apigenin (4′,5,7-trihydroxyflavone) is abundant in many dietary vegetables, fruits and beverages, such as grapes, oranges, apples, parsley, onions, and red wine (Budhraja et al. 2012). Apigenin was also reported to exert pronounced antioxidant (Papay et al. 2017), anti-inflammatory (Patil et al. 2016), and antibacterial properties (Morimoto et al. 2015).

Morin (3,5,7,2′,4′-pentahydroxyflavone) is abundantly found in many fruits, vegetables, and red wines and has many pharmacological activities such as antioxidant, antidiabetic, anticancer, and anti-inflammatory (Park et al. 2014; Qu et al. 2018; Sinha et al. 2016; Ji et al. 2018; Yao et al. 2017). Morin reportedly inhibits human leukemia HL-60 cells growth by inducing apoptosis through a mitochondria-dependent free radical scavenging pathway, suggesting its anticancer property (Park et al. 2014). The antioxidant potential of morin was also observed in the attenuation of antipsychotic-mediated neurotoxicity (Selvakumar et al. 2012). It is known that the anti-inflammatory activity of morin is mediated by modulation of lipoxygenase-1, inducible nitric oxide synthase, inflammatory cytokines, and cyclo-oxygenase expression in macrophages and mast cells (Kempuraj et al. 2005). Morin showed remarkable protection to mammalian hepatocytes, erythrocytes, and endothelial cells against free radical-induced oxidative stress (Ola et al. 2014; Zhang et al. 2011).

Although these compounds showed higher antioxidant and other pharmacological activities, the detailed mechanisms, and rationale for their antioxidant property are poorly elucidated. It is known that the bioactivity of flavonoids is highly structure dependent and differences in the number and distribution of hydroxyl groups can influence the type of interactions between different flavonoids and lipid bilayers and thereby, affect their bioactivities (Panche et al. 2016). However, limited studies on the structure–activity relationship of flavonols in terms of their membrane interactions are available. Since many biological activities are membrane-dependent, therefore, the study of their structure-dependent ability to interact with the membrane might give a partial rationale for their biological effects and correlate it to their ability to inhibit lipid peroxidation (Verstraeten et al. 2015).

This work aims at investigating the antioxidant activity of these compounds and correlating their activity with their location and orientation in lipid membrane models by MD simulations followed by ITC experiments. Molecular dynamics (MD) simulation is a powerful technique to study the dynamics of biological systems at an atomic level for the quantitative estimations of thermodynamics and free energy descriptions of membrane–compound interaction behaviors at nanosecond time resolutions (Lopes et al. 2017; Ulmschneider et al. 2018). The correlation between antioxidant ability of flavonols and their behavior with biomembranes is thus rationalized in this present work.

Materials and Methods

Chemical Materials

Fisetin, apigenin, morin, and cholesterol (3β)-cholesten-5-en-3-ol) were purchased from Sigma Chemicals (St. Louis, MO, USA). POPC lipid was obtained from Avanti polar lipids (Alabaster, AL). Analytical grade chemicals were procured from Hi Media Laboratories Pvt. Ltd., Mumbai, India. All the compounds were used as received due to their high purity (> 99%) and were stored according to the supplier information. Chloroform and ethyl alcohol (purity > 99%) were obtained from Merck.

Antioxidant Activity

Reducing power, superoxide, nitric oxide, and hydrogen peroxide radicals scavenging activity of the compounds were evaluated according to the methods described in our previous paper (Papay et al. 2017). Percent inhibition and IC_{50} values (concentration required to scavenge 50% of the radicals) were calculated by plotting curves of scavenging activity (%) versus the concentration of compounds (μg/mL) and compared with standard reference compound, ascorbic acid using GraphPad Instat, software, version 3.0.

Molecular Dynamics (MD) Simulations

The most abundant lipid component of mammalian cell membranes constitutes unsaturated phosphatidylcholines (van Meer et al. 2008). In this context, we considered the zwitterionic POPC lipid bilayer model with one *cis* double bond between C9 and C10 in the β-chain. For MD simulations, the lipid bilayer models comprise of 128 molecules of POPC surrounded by 2640 TIP4P water molecules without any cholesterol. For cholesterol-containing membrane model, the lipid bilayer model comprises of 176 molecules of POPC containing 40% cholesterol surrounded by 8920 TIP3 water molecules, respectively. All molecular dynamics simulations were performed with Desmond 2016 MD package (Maestro-Desmond Interoperability Tools, version 10.7, Schrödinger, New York, NY, 2016) using the OPLS-AA 2005 force field (Banks et al. 2005). The solute (Compound: lipid bilayer) was immersed in octahedron box spaced at a buffer size of 1 nm from solute boundaries (Stach et al. 2012). MD simulations were performed with 0.15 M NaCl (physiological concentration of monovalent ions) in 10 Å buffer under standard NPT ensemble in an electro-neutral system by implementing the Berendsen thermostat and barostat methods. MD production run was carried out for 100 ns using minimization, equilibration, and production run. The energy minimization and relaxation of the system was carried out using Steepest Descent and the limited-memory Broyden–Fletcher–Goldfarb–Shanno algorithms in a hybrid manner with a step size of 0.005 ps and switch criteria of 25 kcal mol^{-1} $Å^{-1}$. The short-range interactions used a cutoff radius of 9.0 Å, whereas the long-range coulombic interactions were taken into account using smooth Particle Mesh Ewald (PME) with Ewald tolerance of 1e-09 Å (Essmann et al. 1995).

This was followed by the equilibration step in which 2000 steps were performed for the steepest descent minimization of water molecules and ions to retain a lower energetic geometry, while the solute was restrained with force constant of 50 kcal/mol $Å^2$. The systems were then followed by another 2000 steps of minimization resulting in root mean square gradient of 0.1. This was then followed heating of 12 ps at 10 to 300 K in a constant volume ensemble. After this, constant pressure of 12 ps was applied with an unrestrained simulation at 300 K.

Then the final production runs were performed for 100 ns with the records of coordinates were taken every 4.8 ps and the energies were recorded every 1.2 ps at 300 K using NPγT ensemble and surface tension of 4000 bar Å. The bond lengths to hydrogen were constrained with a variant of the M-SHAKE algorithm (Krautler et al. 2001). The system was maintained during the course of the simulation at a constant temperature of 300 K and 1 atm of pressure by using Martyna–Tobias–Klein Barostat algorithm with a relaxation time of 2 ps with isotropic coupling style and Nose–Hoover thermostat algorithm (with a relaxation time of 1 ps) (Evans and Holian 1985; Martyna 1994). And, partial mesh ewald (PME) algorithm was followed for long-range electrostatic interactions (Essmann et al. 1995). The van der Waals (VDW) and electrostatic interactions were truncated at 10 Å, and estimated the long-range VDW contributions to the energy and the pressure by assuming a homogeneous distribution of VDW spheres with dispersion coefficient 69.5 kcal/mol/Å. RESPA (Reversible Reference System Propagator Algorithms) integrator 17 with steps of 2 fs was used for bonded and short-range non-bonded interactions, and 6 fs for long-range electrostatics.

Analysis of Molecular Dynamic Simulations

All the figures were generated using program PyMOL (DeLano Scientific LLC http://www.pymol.org). The position of phosphate atoms in POPC head groups and the distance along the z-axis of the compounds from the center of the lipid bilayer were used to determine the center of mass of that entity (COM; *d*) and the centers of masses of its corresponding characteristic groups. The average area per lipid was calculated without and with compounds from the product of the X and Y dimension of the MD simulation cell divided by the number of lipids per leaflet (Kollman et al. 2000).

Binding Free Energy Calculations

Molecular mechanics Poisson–Boltzmann surface area equation based on the MM/PBSA method was applied for the estimation of binding free energy of systems (Kollman et al. 2000). The binding free energy was estimated by

$$\Delta G_{binding} = G_{complex} - \left(G_{compound} + G_{bilayer}\right),$$

where $\Delta G_{binding}$ is the total free energy of the compound-bilayer complex, and $G_{compound}$ and $G_{bilayer}$ are total energy of separated compound and bilayer in solvent, respectively. Each energy term, G, is estimated as the sum of the average

molecular mechanics potential energy in vacuum (E_{MM}), and the free energy of solvation ($G_{solvation}$) according to following equation:

$$G_{compound/\,bilayer} = E_{MM} + G_{solvation}$$

The molecular mechanics potential energy was calculated in vacuum as following:

$$E_{MM} = E_{bonded} + E_{non-bonded} = E_{bonded} + \left(E_{vdw} + E_{elec}\right)$$

The term, E_{bonded} is bonded interaction including of bond, angle, dihedral, and improper interactions and $E_{non\text{-}bonded}$ is non-bonded interactions consisting of van der Waals (E_{vdw}) and electrostatic (E_{elec}) interactions. The term, ΔE_{bonded} is always taken as zero. Furthermore, the solvation energy is decomposed into the polar solvation energy (G_{ps}) and the non-polar solvation energy (G_{nps}) according to following equation:

$$G_{solvation} = G_{ps} + G_{nps}$$

The G_{ps} was computed using the Poisson–Boltzmann (PB) equation and G_{nps} was calculated based on the solvent-accessible surface area (SASA), according to (Liu et al. 2011):

$$G_{nps} = \gamma SASA + \beta,$$

where *SASA* was determined using the molsurf method using a probe radius of 1.4 Å, whereas surface tension coefficient γ and fitting parameter constant β. The values of the constants according to the literature are (Liu et al. 2011):

$$\gamma = 0.02267 Kj/Mol/\text{Å}^2 \; or \; 0.00542 Kcal/Mol/\text{Å}^2$$

$$b = 3.849 Kj/Mol \; or \; 0.916 Kcal/Mol$$

Isothermal Titration Calorimetry (ITC)

POPC was dissolved in dichloromethane followed by the evaporation of the solvent under a gentle stream of nitrogen and then subjected to vacuum-desiccation for an appropriate time. The dried POPC was then hydrated with Tris-buffered saline at pH 7.5 and ion strength of 154 mM. ITC measurements were obtained from ITC 200 microcalorimeter (MicroCal, Northampton, MA) at 30 °C. The data obtained were analyzed using the One Set of Sites model of the MicroCal version of ORIGIN 7.0 software. The membrane association coefficients (K_a), standard free energy change ($\Delta G_{insertion}$), enthalpy of insertion ($\Delta H_{insertion}$), and entropy of insertion ($\Delta S_{insertion}$) were calculated according to the standard equations.

Statistical Analysis

The results were expressed as the mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Instat, software, version 5.0. The values were analyzed by one-way Analysis of Variance followed by Tukey's multiple comparison test at a significance level of $p < 0.05$.

Results

Antioxidant Activity of Flavonols

Initially, we investigated the antioxidant activity of selected flavonols, which showed significant radical scavenging activity against superoxide, nitric oxide, and hydrogen peroxide-induced radicals. The reducing ability of the compounds to transform Fe^{3+} to Fe^{2+} was measured in terms of absorbance at various concentrations. The higher absorbance of the reaction mixture indicates the higher reducing power of the compound. As compared with fisetin and apigenin, morin showed highest and dose-dependent increase in reducing ability, with the maximum effect attained at 15 µg/mL ($r^2 = 0.993$, Fig. 1a). Fisetin ($r^2 = 0.985$) and apigenin ($r^2 = 0.970$) also showed significant reducing capacity at similar concentration levels (Fig. 1a).

The ability to scavenge superoxide radicals generated from dissolved oxygen by PMS-NADH coupling was measured in terms of reduction of NBT. In this regard, our study shows that superoxide scavenging activity in the presence of morin was about 79 ± 2.3% ($r^2 = 0.970$), whereas it was about 71 ± 1.9% ($r^2 = 0.978$) and 60.4 ± 1.6% ($r^2 = 0.983$), respectively, in the presence of fisetin and apigenin (Fig. 1b).

Similarly, a significant increase in nitric oxide radical quenching activity of morin was seen in a concentration-dependent manner ($r^2 = 0.960$; Fig. 1c). Although, fisetin ($r^2 = 0.973$) and apigenin ($r^2 = 0.981$) show significant nitric oxide radical scavenging activity, however, their percent inhibition was less as compared with morin at similar concentrations, indicating the stronger capacity of morin (Fig. 1c).

A similar trend was observed for hydrogen peroxide scavenging activity when incubated with these compounds at a concentration range of 2.5 to 15 µg/mL (Fig. 1d). The highest percent inhibition was observed with morin at a concentration of 15 µg/mL (80.4 ± 2.4%; $r^2 = 0.939$; Fig. 1d). This was followed by fisetin ($r^2 = 0.944$) and apigenin, respectively ($r^2 = 0.956$; Fig. 1d).

Location and Orientation of Flavonols in Lipid Bilayer

Here, we studied the interaction of selected flavonols (Fig. S1) with unsaturated (POPC) lipid bilayer with cholesterol

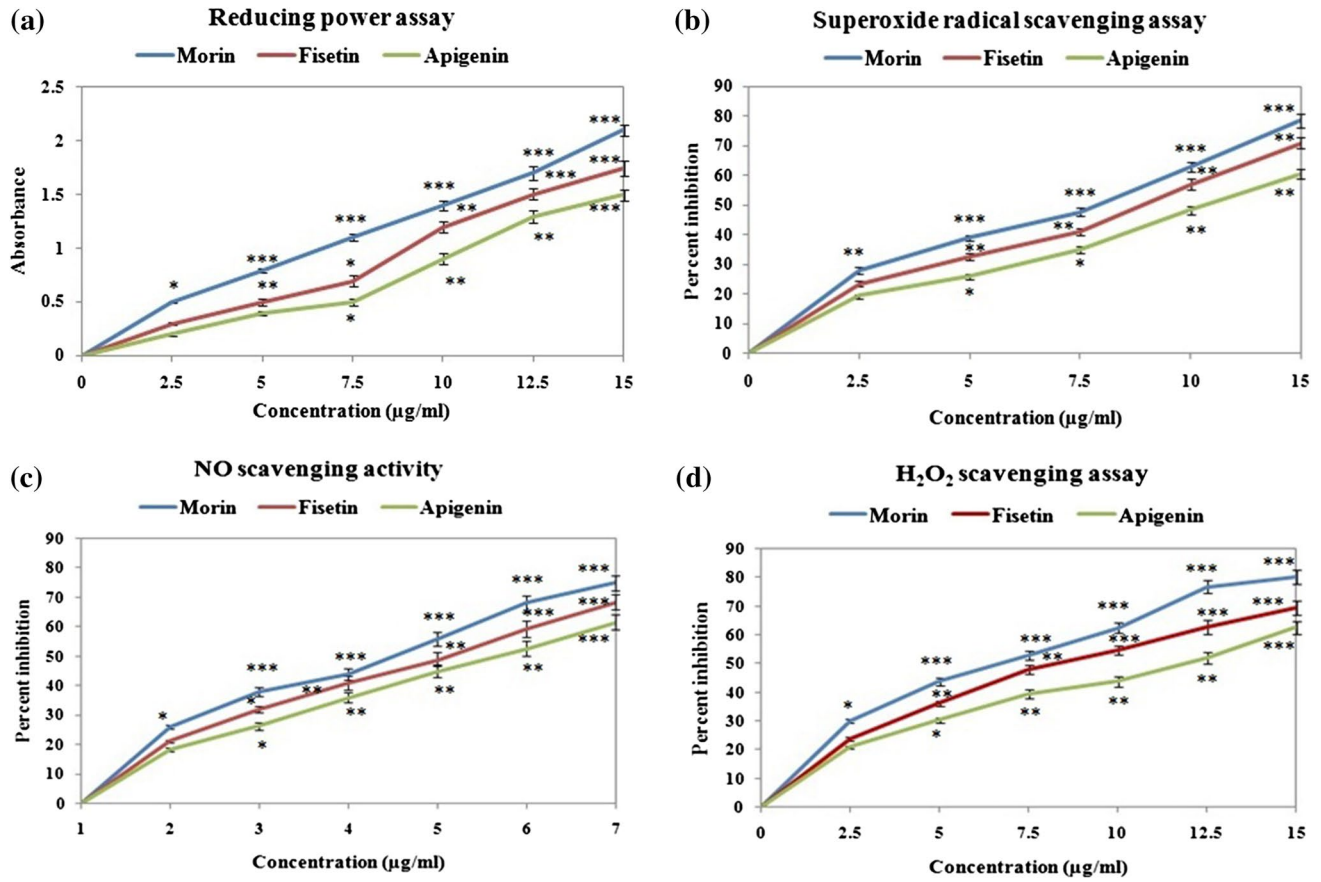


Fig. 1 Antioxidant activity of the selected compounds: **a** reducing power; **b** superoxide radical scavenging; **c** nitric oxide radical scavenging; and **d** hydrogen peroxide radical scavenging assays. Data are presented as mean ± SEM of triplicates. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ from 0 µg/mL extract/ascorbic acid

(0 and 40%) by means of MD simulations. Presently, we focused on the location and orientation of the selected flavonols in the lipid bilayers and the effect of them on the quantitative properties of the lipid bilayers.

Initially, we place all the compounds in the interior of the lipid bilayer phase (Fig. 2a, c, and e). The final snapshots of all MD simulations showed that the compounds incorporates into the lipid bilayer and finally equilibrates between hydrophobic core and polar surface of lipid bilayer (Fig. 2b, d and f). The results indicate that morin approaches towards the surface of the lipid bilayer within 25 ns and then finally relaxes at the membrane surface at the water–lipid interface through the formation of hydrogen bonds after 100 ns (Fig. 2f). Fisetin and apigenin also orient in a similar kind of location at the membrane surface. In case of morin, the hydroxyl groups of A and C ring orient towards the lipid-water phase and forming H-bonds with the water molecules, whereas the B ring orients itself towards the inside of the lipid bilayer forming H-bonds with the oxygen atoms in the glycerol and phosphate groups in the lipid head groups (Fig. 2b and c). The 2′-OH interacts with polar head groups of lipid bilayer by forming H-bonds, whereas 4′–OH, 3–OH, 5–OH, and 7–OH interact with water molecules via H-bonds at the polar surface of the membrane. The 2′–OH was oriented towards the lipid bilayer and thus responsible for anti-lipid peroxidation effect, whereas OH groups at C-3, C-5, and C-7 along with C-4 are free radical scavenger active groups responsible for the antioxidant activity of the morin. The average position of the center of mass of morin after 100 ns was found to be at 1.8 ± 0.18 nm (Table 1).

Apigenin locates itself in somewhat parallel orientation with the lipid bilayer closer to the polar head groups of the bilayer, the OH group of the B ring is the sole H-atom donor which is responsible for the H-bonding interactions between this group and both the polar head groups of the bilayer and the water molecules (Fig. 2c and d). This results in the formation of a water cavity due to the deformation of the membrane surface which favors the formation of H-bonds. The OH- groups of A and C rings of apigenin are nearly at a similar distance from the center of the bilayer, indicating almost the parallel orientation of the compound to the surface. In our study, unlike apigenin, fisetin locates itself

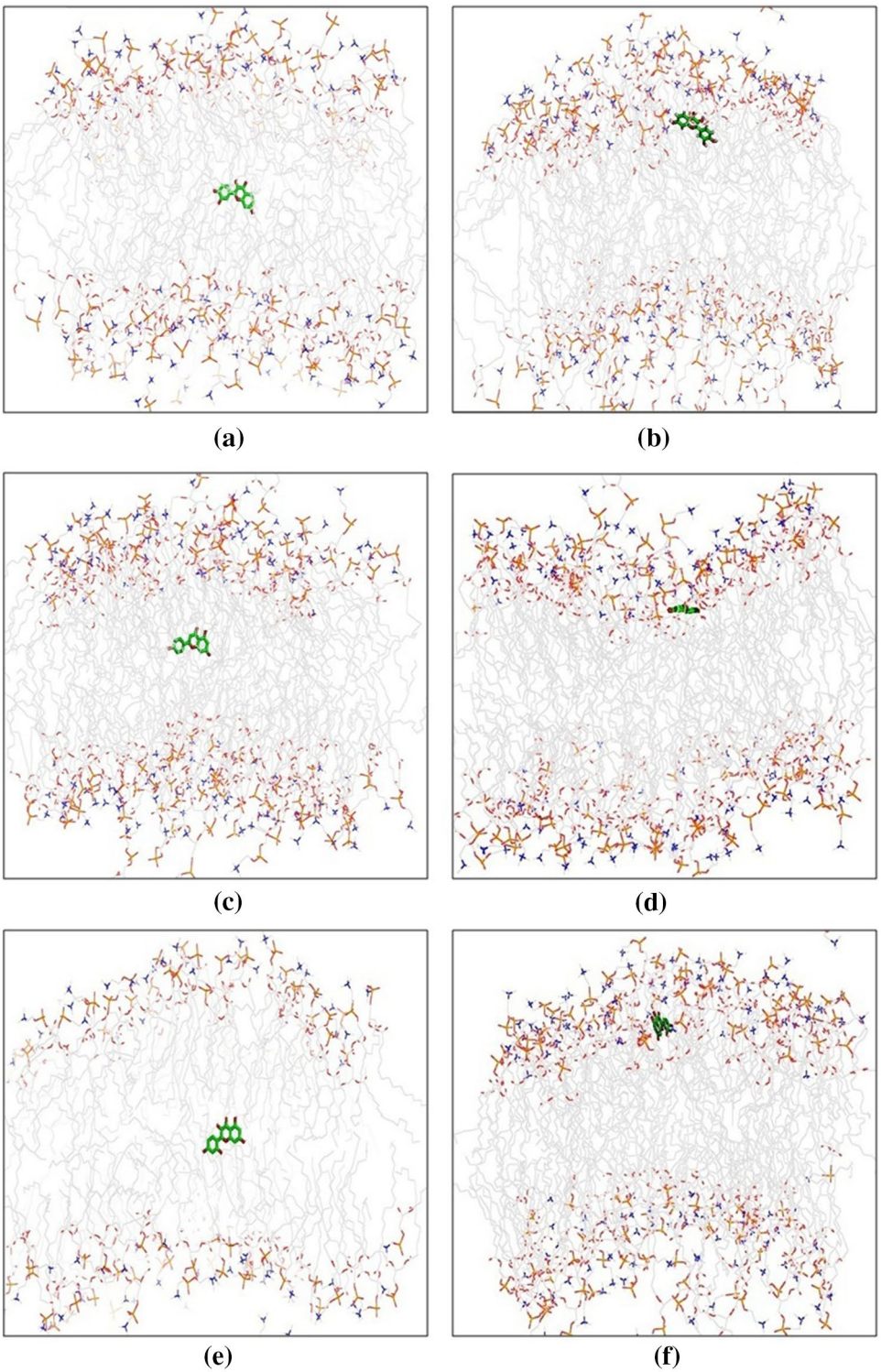


Fig. 2 Snapshots of initial and last frame of MD simulations in the POPC lipid bilayer characteristic to the location **a**, **b** fisetin, **c**, **d** apigenin and **e**, **f** morin, respectively. The water molecules have been removed for sake of clarity. Red and purple color denotes oxygen and phosphorus atoms, respectively (Color figure online)

in somewhat tilted orientation in the lipid bilayer with B-ring hydroxyl groups at the 3′ and 4′ positions are pointing towards the inside of the lipid bilayer (Fig. 2a and b). The average position of the center of mass for fisetin and apigenin were found to be at 1.7 ± 0.15 and 1.9 ± 0.14 nm, respectively (Table 1).

Figure 3 shows snapshots of the initial and final configurations illustrating the location and the orientation of the molecules in the POPC bilayer membrane with 40% cholesterol. The change in location and orientation among the compounds could be related to the intermolecular interactions with the cholesterol. The orientation of apigenin and fisetin

Table 1 Average distances *d* (nm) from the center of lipid bilayer

Compounds	3OH	5OH	7OH	3′OH	4′OH	2′OH	COM
POPC							
Fisetin	1.8 ± 0.17	–	1.65 ± 0.12	1.5 ± 0.11	1.6 ± 0.14	–	1.7 ± 0.15
Apigenin	–	1.96 ± 0.18	1.98 ± 0.15	–	2.1 ± 0.17	–	1.9 ± 0.14
Morin	1.87 ± 0.15	1.9 ± 0.17	1.7 ± 0.18	–	1.79 ± 0.19	1.73 ± 0.16	1.8 ± 0.18
POPC/Cholesterol (40%)							
Fisetin	2.3 ± 0.18	–	2.1 ± 0.16	2.14 ± 0.2	2.23 ± 0.22	–	2.3 ± 0.19
Apigenin	–	2.6 ± 0.21	2.5 ± 0.18	–	2.35 ± 0.17	–	2.4 ± 0.21
Morin	2.05 ± 0.15	2.2 ± 0.18	2.3 ± 0.14	–	1.90 ± 0.12	1.98 ± 0.11	2.1 ± 0.17

Simulations were replicated five times independently

All values were represented as mean ± SEM

differs from that of morin, however, all of them interacted with the lipid head groups on the bilayer surface (Fig. 3). The latter corresponds to perpendicular orientation of the A and B rings with respect to the surface of lipid bilayer. The A (5–OH and 7–OH) and B rings (3–OH and 4–CO) of morin locate close to the carbonyl groups of POPC. The average positions of the center of mass for fisetin and apigenin were found to be at 2.3 ± 0.19 and 2.4 ± 0.21 nm, respectively (Table 1; Fig. 3e and f).

Morin was located at around 2.1 ± 0.17 nm from the center of the POPC lipid bilayer (Table 1). In fisetin, 3′-OH and 4′-OH were located at 1.5 ± 0.11 and 1.6 ± 0.14 nm, respectively, in PC membrane, whereas in case of cholesterol-containing PC membrane, same 3′–OH and 4′–OH groups were located at 2.14 ± 0.2 and 2.23 ± 0.22 nm, respectively (Table 1; Fig. 3a and b). A similar trend was followed for 4′-OH group in apigenin (Table 1; Fig. 3c and d). MD simulation snapshots that illustrate the major geometries of compounds interactions of lipid bilayer models are presented in the Supplementary material, Fig. S2.

Area Per Lipid and Thickness Profiles

In MD simulation studies, area per lipid and membrane thickness are two most important quantitative structural properties. The area per lipid in the presence of fisetin was increased to 0.664 nm^2 as compared with the POPC alone with initial area per lipid of 0.647 nm^2 (Fig. 4a and b). This was coupled with a decrease in calculated thickness from an initial POPC system of 4.431 to 4.247 nm (Fig. 4a and b). The main part of the molecule was oriented towards the lipid bilayer, however, A ring attracts the molecule to the water phase, thus the calculated change in thickness and area per lipid (Fig. 2a). In the case of apigenin, there was a slight decrease in membrane thickness with a sizeable increase in the area per lipid (4.316 nm and 0.655 nm^2; Fig. 4a and b). The area per lipid was calculated with morin in POPC bilayer system and was observed to be increased by 4% with an average value of 0.671 nm^2, whereas thickness was decreased to 4.158 nm (Fig. 4a and a).

MD simulation of the PC lipid membrane containing 40% cholesterol converged to give an area per lipid of 0.562 nm^2 with a concomitant increase in thickness to 4.621 nm (Fig. 4a and b). In the case of morin, simulation results correlate with the calculations of quantitative bilayer properties showing that area per lipid was decreased to 0.582 nm^2 as compared with pure PC lipid membrane system without cholesterol, however, it has been increased if we compared it with pure PC with 40% cholesterol membrane system (Fig. 4a and b). As compared with PC with 40% cholesterol membrane system, morin showed significant decrease in membrane thickness to 4.325 nm (Fig. 4b).

Hydrogen Bond and Free Energy Profiles of the Molecules–Bilayers Interactions

As depicted in Fig. 5, morin formed higher H-bonds in POPC lipid bilayer with 40% cholesterol per nanosecond as compared with PC bilayer without cholesterol. A similar trend was also followed by fisetin and apigenin. The H-bonds formed by morin increased from 0 to 7 in POPC lipid bilayer without cholesterol (Fig. 5a), whereas in PC membrane bilayer with 40% cholesterol, it increased from 0 to 8 per nanosecond (Fig. 5b). In case of a pure POPC lipid bilayer with 0% cholesterol, flavonols formed H-bonds with water and lipid oxygens as it approached the bilayer (Fig. 5a). However, in the case of POPC lipid bilayer with 40% cholesterol, flavonols formed H-bonds with POPC, cholesterol, and water molecules in the bilayers (Fig. 5b). The average H-bonds formed by morin, fisetin, and apigenin were calculated to be 4.90 ± 0.35, 4.40 ± 0.30, and 3.95 ± 0.17, respectively, for POPC lipid bilayer without cholesterol (Fig. 6). However, in POPC lipid bilayer with 40% cholesterol, the average H-bonds formed by morin, fisetin, and apigenin were calculated to be 5.50 ± 0.23, 5.10 ± 0.21 a,nd 4.70 ± 0.20, respectively (Fig. 6).

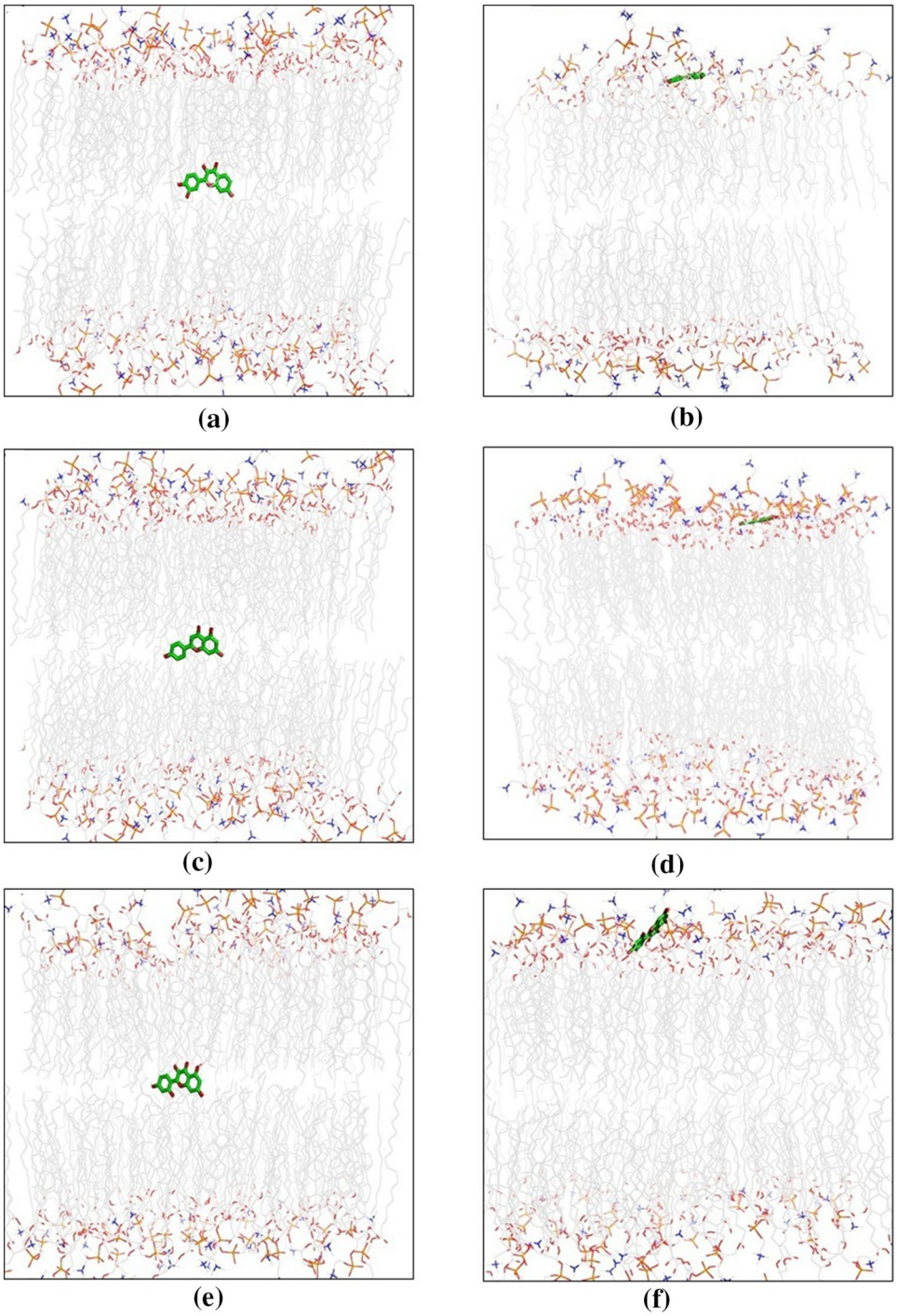


Fig. 3 Snapshots of initial and last frame of MD simulations in the POPC lipid bilayer with 40% cholesterol characteristic to the location **a**, **b** fisetin, **c**, **d** apigenin, and **e**, **f** morin, respectively. The water molecules have been removed for sake of clarity. Red and purple color denotes oxygen and phosphorus atoms, respectively (Color figure online)

Furthermore, morin formed higher number of H-bonds than fisetin and apigenin, which is due to the higher number of OH groups in the morin than that of others (Fig. 6). This was observed in both the lipid bilayer systems. There was a steep rise in H-bonds after almost 25 ns by morin in pure POPC bilayer without cholesterol system. However, in POPC bilayer with 40% cholesterol system, the rise in H-bonds appears after almost 10 ns.

The calculated interactions and overall binding free energies obtained from the MM/PBSA calculations are listed in Table 2. The binding free energy for fisetin in the PC membrane bilayer with 0% and 40% cholesterol was found to be − 112.41 ± 10.44 and − 95.45 ± 10.17 kcal/mol, respectively, which was more than that of apigenin with − 93.88 ± 11.29 and − 70.95 ± 9.13 kcal/mol, respectively (Table 2). Morin showed highest calculated free energy minimum

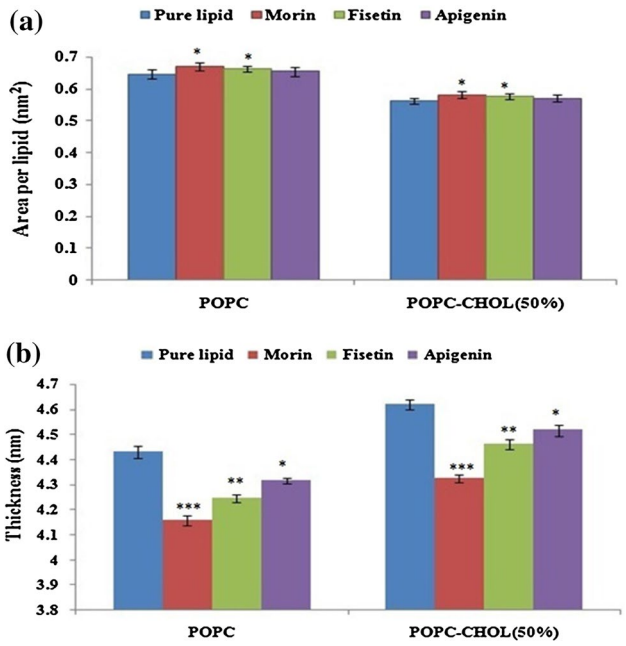


Fig. 4 Quantitative properties of the simulated POPC lipid bilayer with 0% and 40% cholesterol systems **a** Area per lipid and **b** Bilayer thickness. Simulations were replicated five times independently. All values were represented as mean ± SEM. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ from pure lipid

with − 123.23 ± 11.23 and − 111.15 ± 11.85 kcal/mol, respectively, in the PC membrane bilayer with 0% and 40% cholesterol (Table 2).

Isothermal Titration Calorimetry (ITC)

Since morin showed significant antioxidant activity in wet-lab experiments and deepest penetration as well as a highest affinity for POPC lipid bilayer, therefore, we performed ITC measurements to quantify the thermodynamic properties of morin insertion into POPC lipid bilayer. The data of the results for the morin molecule insertion into POPC bilayer are shown in Table 3.

Discussion

The flavonol compounds are well known for various pharmacological activities, however, their mechanism of action with respect to their structure has remained unclear. In this study, all the flavonols (morin, apigenin, and fisetin) were shown to have significant free radical scavenging activity. The high antioxidant activity and inhibition of lipid peroxidation of flavonols could be due to the interaction with cell membranes (Kumar and Pandey 2013). It should be noted that all three compounds are known to act as antioxidants (Markovic et al. 2012; Prasath and Subramanian 2013; Shukla and Gupta 2010).

The differences in the location and orientation of different flavonols depend on the number and distribution of hydroxyl groups and the polymerization degree (Verstraeten et al. 2015). All the selected compounds are amphipathic molecules characterized by the presence of hydrophobic aromatic rings and hydroxyl groups that can donate hydrogen atoms, influencing the type of interactions with lipid bilayer systems. Since these compounds are not expected to penetrate the plasma membrane, therefore, we did 100 ns timescale atomistic MD simulations to quantify the interactions of these compounds with model lipid bilayers. These results are in agreement with wet-lab experiments which showed a protective effect of morin against radiation-induced membrane lipid peroxidation (Zhang et al. 2011).

The 3-OH and 7-OH groups of apigenin are forming H-bonding interactions with the water molecules at the membrane surface and at the nearly same distance as the P-atoms of the lipid, which shows that it does not participate

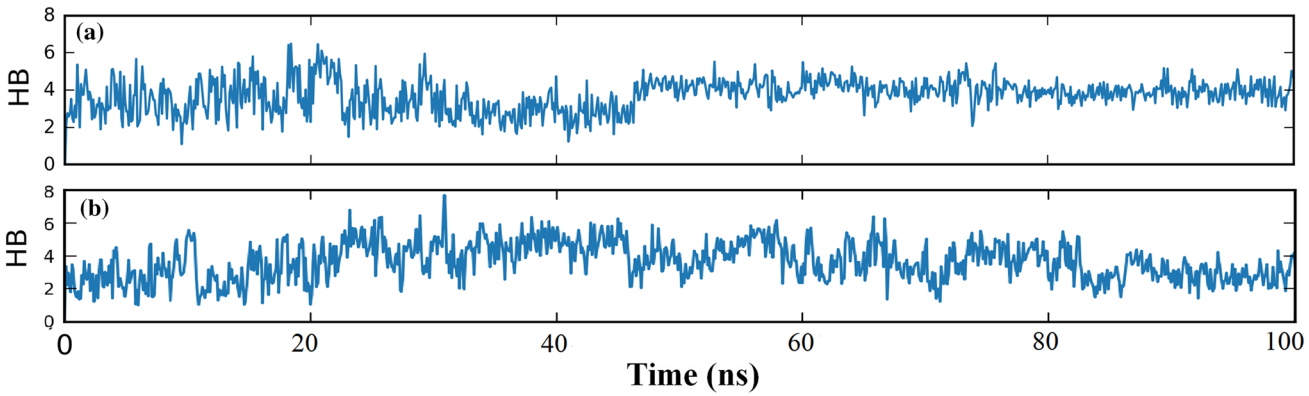


Fig. 5 H-bonds (HB) formed between hydroxyl donors of morin in POPC lipid bilayer with **a** 0% and **b** 40% cholesterol during the 100 ns simulation

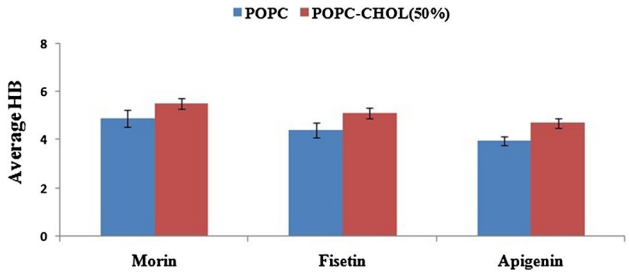


Fig. 6 Average H-bonds formed between fisetin, apigenin, and morin in POPC lipid bilayer with 0% and 40% cholesterol during the 100 ns molecular dynamics simulation. Simulations were replicated five times independently. All values were represented as mean ± SEM

in the scavenging of lipid peroxyl radicals (LOO•). This kind of tendency was in agreement with a recent study which showed that apigenin induces a rearrangement of the DPPC lipid bilayer and thus, increasing its permeability (Kocábováa et al. 2018).

The 3′-OH and 4′-OH of B ring in case of fisetin and 4′-OH of apigenin are pointing towards the solvent and polar head groups of the lipid bilayer and orients itself in parallel orientation at the membrane surface. The scavenger active hydroxyl groups 3′-OH and 4′-OH interact as H-donors with lipid oxygen atoms as H-acceptors to form H- bonds with the phosphate oxygen atoms. Since, the molecules remain at the membrane surface, thus definitely cannot scavenge LOO• free radicals. However, the partitioning and permeability of molecules into cholesterol-containing PC membrane decreases with 40% cholesterol content. The decrease in permeability of molecules could be correlated with the increase of the phospholipid surface density (De Young and Dill 1988).

The present data suggested that all the molecules permeate deeply into the inside of the PC lipid bilayer, whereas they only contacted with the surface with limited sites in cholesterol-containing PC lipid bilayer. Thus, the partitioning of molecules was more sensitive to cholesterol. According to a previous study, quercetin interacts preferentially with cholesterol which leads to the reduction in its availability inside the lipid bilayer and thus, reducing its antioxidant potential (Lupanova et al. 2012).

The membrane thickness is considered as the distance between the center of mass of the phosphorus atoms in the two leaflets of the lipid membrane bilayer. The changes observed were in agreement with the simulation results which indicate that morin occupies some space inside the lipid bilayer at the membrane surface. The presence of an additional hydroxyl group at 2′ position of the B ring in morin is found to be responsible for the change in the orientation. The 3-OH, 5-OH, 3′-OH, and 4′-OH groups of morin are free radical scavenger active groups and found to form H-bonds with lipid head groups and water molecules. Thus, we can conclude that morin can effectively scavenge free radicals flowing across the lipid bilayer and thereby inhibiting lipid peroxidation. However, it will not be an efficient molecule for the scavenging of lipid peroxyl radicals since the active free radical scavenger active groups are far from the center of the bilayer where these radicals are formed. Whereas, in the case of apigenin, an extra hydroxyl group in B ring interacts with lipid oxygen acceptors in particular, O9, O10, and O16 as well as with surrounding water molecules. The 5-OH and 7-OH groups of A ring are mainly responsible for the solvent interactions and therefore could be major active groups only when scavenging hydroxyl radicals, otherwise OH groups in B ring are responsible for antioxidant activity of the molecule. It is expected that apigenin would be less efficient than morin and fisetin in scavenging LOO• radicals. These results are in agreement with experimental results which showed that 5-hydroxyflavones

Table 3 Thermodynamic data for partitioning of the morin from water into POPC vesicles (303 k)

DMPC	$\Delta G_{insertion}$ (Kcal.M^{-1})	$\Delta H_{insertion}$ (Kcal.M^{-1})	$\Delta S_{insertion}$ (cal. $M^{-1}.K^{-1}$)	K_a (M^{-1})
Morin	− 22.96	− 5.99	56	9106

Table 2 Average MM/PBSA free energies of compound-bilayer complexes calculated from the MD simulations performed in quintuplicate

Energy (kcal/mol)	POPC			POPC/Cholesterol (40%)		
	Fisetin	Apigenin	Morin	Fisetin	Apigenin	Morin
ΔE_{ELE}	− 216.80 ± 13.17	− 205.75 ± 10.41	− 223.78 ± 12.56	− 187.38 ± 11.43	− 165.41 ± 9.62	− 206.52 ± 11.03
ΔE_{VDW}	− 182.53 ± 11.26	− 162.48 ± 10.14	− 193.55 ± 11. 95	− 163.40 ± 10.29	− 148.90 ± 12.56	− 174.30 ± 10.80
ΔE_{GAS}	− 399.33 ± 14.79	− 368.23 ± 13.12	− 417.33 ± 14.28	− 350.78 ± 12.81	− 314.31 ± 12.75	− 380.82 ± 13.25
ΔG_{NPS}	− 27.90 ± 7.24	− 21.50 ± 12.56	− 30.60 ± 5.70	− 22.47 ± 7.85	− 19.07 ± 6.26	− 25.70 ± 8.05
ΔG_{PS}	314.82 ± 12.42	295.85 ± 12.56	324.70 ± 17.29	277.80 ± 11.92	262.43 ± 10.80	295.37 ± 14.29
ΔG_{SOL}	286.92 ± 11.85	274.35 ± 10.97	294.10 ± 16.48	255.33 ± 12.68	243.36 ± 11.97	269.67 ± 12.32
$\Delta G_{binding}$	− 112.41 ± 10.44	− 93.88 ± 11.29	− 123.23 ± 11.23	− 95.45 ± 10.17	− 70.95 ± 9.13	− 111.15 ± 11.85

Results were shown as the mean ± SEM of five simulations

including morin and fisetin are more potent in lipid peroxidation inhibition than 3-hydroxyflavones including apigenin (Sugihara et al. 1999).

A similar trend was followed with all the molecules in PC lipid bilayer with 40% cholesterol when compared with their counterparts in pure PC lipid membrane systems. These results indicated that molecules get diffuse from the lipid bilayer to the surrounding aqueous phase when in PC with 40% cholesterol bilayer system. These changes were in agreement with earlier studies which showed that the decrease in permeability of molecules in cholesterol-containing PC membranes is correlated with the increase of the phospholipid surface density (Patil et al. 2016). Recently, it has been suggested that this correlation is because of the area-dependent resistance at the lipid head groups and alterations in the partition coefficient (Shinoda 2016).

Since intermolecular interactions play an important role in the penetration and permeability of compounds on the membrane lipid bilayer surface, therefore, we also studied the hydrogen bonding between the hydroxyl groups of the flavonols with the lipid oxygens (Sirk et al. 2011). The hydroxyl groups of the flavonols, cholesterol, and water molecules were considered as both hydrogen bond donors and acceptors. For this, we performed a time-dependent analysis of the H-bonds between them. As predicted from the simulations, the flavonols were localized on the bilayer–water interface, therefore, they have the ability to form hydrogen bonds with the water molecules. These data suggested that the kind of orientation of compounds is driven by hydrogen-bonding interactions with the polar head of lipid bilayer as well as with cholesterol molecules. Our results indicated the absorption and deeper penetration of morin inside both the lipid bilayers. Furthermore, inside the bilayer, all the hydroxyl groups become accessible to the lipid oxygens which apparently increase the number of H-bonds. This was due to the fact that the location of morin was at water–bilayer interface and OH groups of morin were involved in H-bonding with cholesterol molecules as well as with water and lipid bilayer. Similar kind of increase in H-bonds with PC membrane bilayer by the incorporation of cholesterol was also observed in a previous computational study (Karamia and Jalili 2014).

Apart from the change in the membrane structural properties, binding free energy profiles were also affected by the cholesterol content in the PC membrane bilayer. To obtain insight about the overall binding free energies, we have calculated interactions such as van der Waals, electrostatic, polar solvation energy, and non-polar solvation energy using MM/PBSA method (Xu and Wang 2006). It has already been showed that the higher negative values of the binding free energies signify stronger affinity of the ligand to the membrane lipid bilayer (Ossman et al. 2016). The changes in binding free energy of morin were because of the higher energetic cost required for deeper penetration towards the inside of the PC bilayer without cholesterol. This localization of morin reduced the repulsive forces among the phospholipid head groups, thus decreasing the mobility of the hydrocarbon chains (Reiner et al. 2013). As depicted in Table 2, both van der Waals and electrostatic interactions of morin in POPC bilayer with 0% and 40% cholesterol were higher than that of other related molecules, suggesting that van der Waals and electrostatic interactions also contributed significantly to the affinity of morin towards the membrane bilayers. Furthermore, the electrostatic interactions of morin were also lower than that of fisetin and apigenin, respectively, for POPC bilayer with 0% and 40% cholesterol, resulting in a lower binding free energy with the bilayers than that of corresponding compounds. Moreover, from the results of binding free energies, we could also suggest that the decrease in binding free energies of the molecules in POPC bilayer with 40% cholesterol might be due to the reduced permeability of a membrane on the addition of cholesterol (Subczynski et al. 1994; Papahadjopoulos et al. 1972). This could be due to the fact that the double bond in the unsaturated POPC lipid induces a kink in the hydrocarbon chain which precludes tight packing with the planar and rigid cholesterol molecules, leading to more disorder and free volume in the POPC/cholesterol membranes and resulting in a slower increase of binding free energies (Pandit et al. Pandit et al. 2008). Interestingly, the sequence of effects on the bilayer properties induced by flavonols correlates with the binding free energies and the sequence was in the order of morin > fisetin > apigenin. This could be explained by the fact that more pronounced charge may pull the compounds out of the bilayers due to their location at the water–bilayer interface and thereby, may be less efficient in lipid peroxidation inhibition activity. The other computational studies on the influence of cholesterol on lipid bilayers also correlate with our findings (Sanver et al. 2016; Lopes et al. 2017; Wennberg et al. 2012).

However, taken together, all results suggest that morin has shown the most significant antioxidant activity against the damage caused by free radicals. Thus, we extended our investigations to determine the mechanism of morin into the lipid bilayer by ITC experiments. The ITC data suggested that morin molecule partitioned spontaneously into the POPC membrane as predicted by the negative $\Delta G_{insertion}$. The results also suggest that the insertion of morin is driven mostly by an increase in entropy. Since morin is non-polar, its insertion into the bilayer is driven by the hydrophobic effect with minimal enthalpic interactions with the headgroups (Table 3).

Conclusions

In conclusion, we found that although all the three compounds (Morin, fisetin, and apigenin) have significant antioxidant activity, however, morin showed most potent results among them which could be due to the differences in their structure. We analyzed the influence of cholesterol, hydroxyl groups in flavonols, substituted OH group and their intermolecular H-bonding ability on the location, orientation, and the penetration depth of flavonols in lipid bilayer. All the compounds incorporate into the POPC lipid bilayers, however, morin showed the deepest penetration into the lipid bilayer which might be attributed to the hydroxyl groups at position 3 as well as 5. Our results rationalize the higher efficiency of morin as compared with fisetin and apigenin to scavenge $LOO^{\bullet}$ radicals as well as to interrupt hydroxyl radicals which used to generate in the water phase, diffuse easily across the lipid bilayer and causing oxidation of acyl chains. In addition, the incorporation of cholesterol in the lipid bilayer system can delay or prevent the deeper penetration of the flavonols which might be due to the H-bonding with the cholesterol at the polar interfacial region of the lipid bilayer. We strongly believe that the cholesterol in POPC lipid bilayer could result in a reduction in the area per lipid values and a corresponding rise in the bilayer thickness. The MD results were in agreement with the ITC experimental results. Thus, this study shows how the positioning of compounds in lipid membrane could affect its antioxidant properties as well as also contributed an understanding of permeability across membranes foundation for the encapsulation of these compounds into liposomes. However, further detailed experimental studies are required to investigate the effect of cholesterol in membranes of saturated and polyunsaturated lipids.

Acknowledgements We sincerely thank Indian Institute of Technology Delhi HPC facility for computational resources.

Compliance with Ethical Standards

Conflict of interest The authors state that there are no conflicts of interests.

Research Involving Human and Animal Participants This article does not contain any studies with human or animal subjects.

References

Aguilera Y, Martin-Cabrejas MA, de Mejia EG (2016) Phenolic compounds in fruits and beverages consumed as part of the mediterranean diet: their role in prevention of chronic diseases. Phytochem Rev 15:405–423

Ahmad A, Ali T, Park HY, Badshah H, Rehman SU, Kim MO (2017) Neuroprotective effect of Fisetin Against amyloid-beta induced cognitive/synaptic dysfunction, neuroinflammation, and neurodegeneration in adult mice. Mol Neurobiol 54:2269–2285

Asgar A (2013) Anti-diabetic potential of phenolic compounds: a review. Int J Food Prop 16:91–103

Banks JL, Beard HS, Cao Y, Cho AE, Damm W, Farid R, Felts AK, Halgren TA, Mainz DT, Maple JR, Murphy R, Philipp DM, Repasky MP, Zhang LY, Berne BJ, Friesner RA, Gallicchio E, Levy RM (2005) Integrated modeling program, applied chemical theory (IMPACT). J Comput Chem 26:1752

Budhraja A, Gao N, Zhang Z, Son Y, Cheng S, Wang X, Ding S, Hitron A, Chen G, Luo J, Shi X (2012) Apigenin induces apoptosis in human leukemia cells and exhibits anti-leukemic activity in vivo. Mol Cancer Ther 11:132–142

D'Andrea G (2015) Quercetin: a flavonol with multifaceted therapeutic applications? Fitoterapia 106:256–271

Davatgaran-Taghipour Y, Masoomzadeh S, Farzaei MH, Bahramsoltani R, Karimi-Soureh Z, Rahimi R, Abdollahi M (2017) Polyphenol nanoformulations for cancer therapy: experimental evidence and clinical perspective. Int J Nanomed 12:2689–2702

De Young LR, Dill KA (1988) Aggregation and denaturation of apomyoglobin in aqueous urea solutions. Biochemistry 27:5281–5289

Essmann U, Perera L, Berkowitz ML, Darden T, Lee H, Pedersen LG (1995) A smooth particle mesh Ewald method. J Chem Phys 103:8577–8593

Evans DJ, Holian BL (1985) The Nose-Hoover thermostat. J Chem Phys 83:4069–4074

Fu CY, Chen MC, Tseng YS, Chen MC, Zhou Z, Yang JJ, Lin YM, Viswanadha VP, Wang G, Huang CY (2019) Fisetin activates Hippo pathway and JNK/ERK/AP-1 signaling to inhibit proliferation and induce apoptosis of human osteosarcoma cells via ZAK overexpression. Environ Toxicol 34:902–911

Gonçalves S, Romano A (2017) Inhibitory properties of phenolic compounds against enzymes linked with human diseases, Chapter 6. Phenolic compounds-biological activity. IntechOpen, London

Gurer-Orhan H, Ince E, Konyar D, Saso L, Suzen S (2018) The role of oxidative stress modulators in breast cancer. Curr Med Chem 25:4084–4101

Haddad AQ, Venkateswaran V, Viswanathan L, Teahan SJ, Fleshner NE, Klotz LH (2006) Novel antiproliferative flavonoids induce cell cycle arrest in human prostate cancer cell lines. Prostate Cancer Prostatic Dis 9:68–76

Hanneken A, Lin FF, Johnson J, Maher P (2006) Flavonoids protect human retinal pigment epithelial cells from oxidative-stress-induced death. Invest Ophthalmol Vis Sci 47:3164–3177

Higa S, Hirano T, Kotani M, Matsumoto M, Fujita A, Suemura M, Kawase I, Tanaka T (2003) Fisetin, a flavonol, inhibits TH2-type cytokine production by activated human basophils. J Allergy Clin Immunol 111:1299–1306

Ji Y, Jia L, Zhang Y, Xing Y, Wu X, Zhao B, Zhang D, Xu X, Qiao X (2018) Antitumor activity of the plant extract morin in tongue squamous cell carcinoma cells. Oncol Rep 40:3024–3032

Karamia L, Jalili S (2014) Effects of cholesterol concentration on the interaction of cytarabine with lipid membranes: a molecular dynamics simulation study. J Biomol Struct Dyn 33:1254–1268

Kashyap D, Sharma A, Sak K, Tuli HS, Buttar HS, Bishayee A (2018) Fisetin: a bioactive phytochemical with potential for cancer prevention and pharmacotherapy. Life Sci 194:75–87

Kempuraj D, Madhappan B, Chirstodoulou S, Boucher W, Cao J, Papadopoulou N, Cetrulo CL, Theoharides TC (2005) Flavonoids inhibits pro-inflammatory mediator release, intracellular calcium ion levels, and protein kinase C theta phosphorylation in human mast cells. Br J Pharmacol 145:934–944

Kocábováa J, Kolivoškaa V, Gálb M, Sokolová R (2018) Tuning phospholipid bilayer permeability by flavonoid apigenin: electrochemical and atomic force microscopy study. J Electroanal Chem 221:67–72

Kollman PA, Massova I, Reyes C, Kuhn B, Huo S, Chong L, Cheatham TE (2000) Calculating structures and free energies of complex molecules: combining molecular mechanics and continuum models. Acc Chem Res 33:889–897

Krautler V, Gunsteren WFV, Hunenberger PH (2001) A fast SHAKE: algorithm to solve distance constraint equations for small molecules in molecular dynamics simulations. J Comp Chem 22:501–508

Kumar S, Pandey AK (2013) Chemistry and biological activities of flavonoids: an overview. Sci World J. https://doi.org/10.1155/2013/162750

Liu FF, Dong XY, He L, Middelberg APJ, Sun Y (2011) Molecular insight into conformational transition of amyloid β-peptide 42 inhibited by (−)-epigallocatechin- 3-gallate probed by molecular simulations. J Phys Chem B 115:11879–11887

Lopes D, Jakobtorweihen S, Nunes C, Sarmento B, Reis S (2017) Shedding light on the puzzle of drug-membrane interactions: experimental techniques and molecular dynamics simulations. Prog Lipid Res 65:24–44

Lupanova T, Petkova D, Markovska T, Staneva G, Chakarov S, Skrobanska R, Pankov R, Momchilova A (2012) Effect of cholesterol modulation on the antioxidant potential of quercetin in rat liver plasma membranes. Compt Rend Acad Bulg Sci 65:639–644

Maher P, Akaishi T, Abe K (2006) Flavonoid fisetin promotes ERK-dependent long-term potentiation and enhances memory. Proc Natl Acad Sci USA 103:16568–16573

Mao Z, Gan C, Zhu J, Ma N, Wu L, Wang L, Wang X (2017) Anti-atherosclerotic activities of flavonoids from the flowers of *Helichrysum arenarium L.* MOENCH through the pathway of anti-inflammation. Bioorg Med Chem Lett 15:2812–2817

Markovic Z, Milenkovic D, Dorovic J, Markovic JMD, Stepanic V, Lucic B, Amic D (2012) Free radical scavenging activity of morin 2′-O(-) phenoxide anion. Food Chem 135:2070–2077

Martyna GJ (1994) Remarks on constant-temperature molecular dynamics with momentum conservation. Phys Rev E 50:3234–3236

Morimoto Y, Baba T, Sasaki T, Hiramatsu K (2015) Apigenin as ananti-quinolone-resistance antibiotic. Int J Antimicrob Agents 46:666–673

Neuvonen M, Manna M, Mokkila S, Javanainen M, Rog T, Liu Z, Bittman R, Vattulainen I, Ikonen E (2014) Enzymatic oxidation of cholesterol: properties and functional effects of cholestenone in cell membranes. PLoS ONE 9:e103743

Ola MS, Aleisa AM, Al-Rejaie SS, Abuohashish HM, Parmar MY, Alhomida AS, Ahmed MM (2014) Flavonoid, morin inhibits oxidative stress, inflammation and enhances neurotrophic support in the brain of streptozotocin-induced diabetic rats. Neurol Sci 35:1003–1008

Ossman T, Fabre G, Trouillas P (2016) Interaction of wine anthocyanin derivatives with lipid bilayer membranes. Comput Theor Chem 1077:80–86

Pal HC, Sharma S, Elmets CA, Athar M, Afaq F (2013) Fisetin inhibits growth, induces G(2)/M arrest and apoptosis of human epidermoid carcinoma A431 cells: role of mitochondrial membrane potential disruption and consequent caspases activation. Exp Dermatol 22:470–475

Panche AN, Diwan AD, Chandra SR (2016) Flavonoids: an overview. J Nutr Sci 5:e47

Pandit SA, Chiu SW, Jakobsson E, Grama A, Scott HL (2008) Cholesterol packing around lipids with saturated and unsaturated chains: a simulation study. Langmuir 24:6858–6865

Papahadjopoulos D, Nir S, Ohki S (1972) Permeability properties of phospholipid membranes: effect of cholesterol and temperature. Biochim Biophys Acta Biomemb 266:561–583

Papay ZE, Kosa A, Boddi B, Merchant Z, Saleem IY, Zariwala MG, Klebovich I, Somavarapu S, Antal L (2017) Study on the pulmonary delivery system of apigenin-loaded albumin nanocarriers with antioxidant activity. J Aerosol Med Pulm Drug Deliv 30:274–288

Park C, Lee WS, Go SI, Nagappan A, Han MH, Hong SH, Kim GS, Kim GY, Kwon TK, Ryu CH, Shin SC, Choi YH (2014) Morin, a flavonoid from moraceae, induces apoptosis by induction of BAD protein in human leukemic cells. Int J Mol Sci 16:645–659

Patil RH, Babu RL, Kumar MN, Kumar KMK, Hegde SM, Nagesh R, Ramesh GT, Sharma SC (2016) Anti-Inflammatory effect of Apigenin on LPS-induced pro-inflammatory mediators and AP-1 factors in human lung epithelial cells. Inflammation 39:138–147

Peng HL, Huang WC, Cheng SC, Liou CJ (2018) Fisetin inhibits the generation of inflammatory mediators in interleukin-1β-induced human lung epithelial cells by suppressing the NF-κB and ERK1/2 pathways. Int Immunopharmacol 60:202–210

Prasath GS, Subramanian SP (2013) Fisetin, a tetra hydroxy flavone recuperates antioxidant status and protects hepatocellular ultrastructure from hyperglycemia mediated oxidative stress in streptozotocin induced experimental diabetes in rats. Food Chem Toxicol 59:249–255

Qu Y, Wang C, Liu N, Gao C, Liu F (2018) Morin exhibits anti-inflammatory effects on IL-1β stimulated human osteoarthritis chondrocytes by activating the Nrf2 signaling pathway. Cell Physiol Biochem 51:1830–1838

Reiner GN, Fraceto LF, de Paula E, Perillo MA, Garcia DA (2013) Effects of Gabaergic phenols on phospholipid bilayers as evaluated by 1H-NMR. J Biomat Nanobiot 4:1–7

Roìg T, Pasenkiewicz-Gierula M, Vattulainen I, Karttunen M (2009) Ordering effects of cholesterol and its analogues. Biochim Biophys Acta 1788:7–121

Romano B, Pagano E, Montanaro V, Fortunato AL, Milic N, Borrelli F (2013) Novel insights into the pharmacology of flavonoids. Phytother Res 27:1588–1596

Saija A, Scalese M, Lanza M, Marzullo D, Bonina F, Castelli F (1995) Flavonoids as antioxidant agents: importance of their interaction with biomembranes. Free Radic Biol Med 19:481–486

Sanver D, Murray BS, Sadeghpour A, Rappolt M, Nelson AL (2016) Experimental modeling of flavonoid biomembrane interactions. Langmuir 13:13234–13243

Selvakumar GP, Dhanraj V, Krishnamoorthy M, Tamilarasan M (2012) Morin attenuates haloperidol induced tardive dyskinesia and oxidative stress in mice. J Nat Sci Res 2:153–165

Shinoda W (2016) Permeability across lipid membranes. Biochim et Biophys Acta Biomemb 1858:2254–2265

Shukla S, Gupta S (2010) Apigenin: a promising molecule for cancer prevention. Pharm Res 27:962–978

Sinha K, Ghosh J, Sil PC (2016) Morin and its role in chronic diseases. Adv Exp Med Biol 928:453–471

Sirk TW, Friedman M, Brown EF (2011) Molecular binding of black tea theaflavins to biological membranes: relationship to bioactivities. J Agric Food Chem 59:3780–3787

Stach M, Maillard N, Kadam RU, Kalbermatter D, Meury M, Page MGP, Fotiadis D, Darbre T, Reymond J (2012) Membrane disrupting antimicrobial peptide dendrimers with multiple amino termini. Med Chem Commun 3:86–89

Subczynski W, Wisniewska A, Yin J, Hyde J, Kusumi A (1994) Hydrophobic barriers of lipid bilayer membranes formed by reduction of water penetration by alkyl chain unsaturation and cholesterol. Biochem 33:7670–7681

Sugihara N, Arakawa T, Ohnishi M, Furuno K (1999) Anti- and pro-oxidative effects of flavonoids on metal-induced lipid hydroperoxide-dependent lipid peroxidation in cultured hepatocytes loaded with alpha-linolenic acid. Free Radic Biol Med 27:1313–1323

Sun X, Ma X, Li Q, Yang Y, Xu X, Sun J, Yu M, Cao K, Yang L, Yang G, Zhang G, Wang X (2018) Anti–cancer effects of fisetin on

mammary carcinoma cells via regulation of the PI3K/Akt/mTOR pathway: in vitro and in vivo studies. Int J Mol Med 42:811–820

Syed DN, Afag F, Maddodi N, Johnson JJ, Sarfaraz S, Ahmad A, Setaluri V, Mukhtar H (2011) Inhibition of human melanoma cell growth by the dietary flavonoid fisetin is associated with disruption of Wnt/β- catenin signaling and decreased Mitf levels. J Invest Dermatol 131:1291–1299

Ulmschneider JP, Ulmschneider MB (2018) Molecular dynamics simulations are redefining our view of peptides interacting with biological membranes. Acc Chem Res 51:1106–1116

van Meer G, Voelker DR, Feigenson GW (2008) Membrane lipids: where they are and how they behave. Nat Rev 9:112–125

Verstraeten SV, Fraga CG, Oteiza PI (2015) Interactions of flavan-3-ols and procyanidins with membranes: mechanisms and the physiological relevance. Food Funct 6:32–40

Wennberg CL, van der Spoel D, Hub JS (2012) Large influence of cholesterol on solute partitioning into lipid membranes. J Am Chem Soc 134:5351–5361

Xu Y, Wang RA (2006) computational analysis of the binding affinities of FKBP12 inhibitors using the MM-PB/SA method. Prot Struct Funct Bioinfo 64:1058–1068

Yao D, Cui H, Zhou S, Guo L (2017) Morin inhibited lung cancer cells viability, growth, and migration by suppressing miR-135b and inducing its target CCNG2. Tumour Biol 39:1010428317712443

Youns M, Abdel HHW (2017) The natural flavonoid fisetin inhibits cellular proliferation of hepatic, colorectal, and pancreatic cancer cells through modulation of multiple signaling pathways. PLoS ONE 12:e0169335

Zhang R, Kang KA, Kang SS, Park JW, Hyun JW (2011) Morin (2′,3,4′,5,7- pentahydroxyflavone) protected cells against c-radiation-induced oxidative stress. Basic Clin Pharmacol Toxicol 108:63–72

Zhang H, Zheng W, Feng X, Yang F, Qin H, Wu S, Hou DX, Chen J (2019) Nrf2¯ARE signaling acts as master pathway for the cellular antioxidant activity of Fisetin. Molecules 24:E708

Affiliations

Sarmistha Saha[1] · Emiliano Panieri[2] · Sibel Suzen[3] · Luciano Saso[2]

✉ Sarmistha Saha
sarmistha_pharmacol@yahoo.com

[1] Materials Science and Engineering, Indian Institute of Technology Gandhinagar, Palaj, Gandhinagar, Gujarat, India

[2] Department of Physiology and Pharmacology "Vittorio Erspamer, Sapienza University of Rome, Rome, Italy

[3] Faculty of Pharmacy, Ankara University, Ankara, Turkey