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## Molecular docking simulation analysis of the interaction of dietary flavonols with heat shock protein 90

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### Abstract

Hsp90 is a major protein involved in the stabilization of various proteins in cancer cells. The present investigation focused on the molecular docking simulation studies of flavanols as inhibitors of Hsp90 at the high affinity adenosine triphosphate (ATP) binding site and analyzed absorption, distribution, metabolism, excretion and toxicity (ADME-toxicity). The molecular docking analysis revealed that the flavanols showed competitive inhibition with ATP molecule at the active site and enhanced pharmacological parameters.

**Keywords:** flavonols, cancer, molecular docking, Hsp90, ATP binding site

### Introduction

Flavonols, a class of flavonoids that have 3-hydroxy-flavone backbone (3-hydroxy-2-phenylchromen-4-one)<sup>[1-2]</sup>, are widely present in a variety of fruits and vegetables<sup>[3]</sup>. Some preliminary reports showed that flavonoids are involved in the modification of allergens, viruses and carcinogens<sup>[4-5]</sup>. Moreover, several in vitro studies showed that flavonoids also have anti-allergic, anti-inflammatory<sup>[6]</sup>, anti-microbial<sup>[7-8]</sup>, anti-cancer<sup>[9]</sup> and anti-diarrheal activities<sup>[10]</sup>. Additionally, in vitro studies have demonstrated activity of flavonoids against several viruses<sup>[11]</sup>.

Quercetin is usually present in fruits, vegetables, leaves and grains<sup>[12]</sup>. Kaempferol is found in tea, broccoli, *Kaempferia galangal*, and other fruits, vegetables, leaves and grains<sup>[13]</sup>. while maristin is present in grapes,

berries, fruits, vegetables, herbs, as well as other plants and red wine<sup>[14]</sup>.

Hsp90 is an abundant cytosolic chaperone that is involved in the turnover, trafficking and activity of a large number and variety of client proteins such as membrane-associated proteins and soluble protein kinases<sup>[15-16]</sup>. There are several reports of small molecule hsp90 inhibitors that bind to the N-terminal adenosine triphosphate (ATP) binding pocket and inhibit chaperone function of hsp90<sup>[17]</sup>. Hsp90 inhibitors bind to the N-domain ATP-binding pocket and prevent ATP binding, eventually leading to client protein degradation<sup>[24]</sup>. In response to hsp90 inhibition, cancer cells exhibit several types of response, including reversal of transformation, differentiation and apoptosis<sup>[18-19]</sup>. Hsp90 is also secreted in large quantities and found on the surface of cancer cells<sup>[20-21]</sup>. Hsp90 inhibitors

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are currently undergoing clinical evaluation in cancer patients<sup>[22-23]</sup>.

In the present investigation, 3 common dietary flavonols viz. quercetin, kaempferol and myricetin were screened against Hsp90 using *in silico* molecular docking simulation approaches and virtual screening. The molecular docking simulation in this study revealed that quercetin, kaempferol and myricetin exhibited competitive inhibition with ATP molecule at the ATP-binding pocket of Hsp90.

## Materials and methods

### Protein preparation

The 3 dimensional (3D) crystal structure of Hsp90 N-terminal domain bound to ATP (PDB ID: 3T0Z) was retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (<http://www.rcsb.org/>). The crystal structure has a resolution of 2.19 Å. It also has a structural weight of 26,188.49 Da and amino acid length of 288 and contains only a single chain (Chain A). The enzyme was then imported in the Molegro Virtual Docker (MVD)<sup>[25]</sup>. For molecular docking purpose, all the water molecules were removed because they were considered during the scoring.

### Chemical structures

The 2D structures of quercetin (CID5280343), kaempferol (CID5280863), myricetin (CID5281672) and ATP (CID5957) were retrieved from the NCBI PubChem database<sup>[26]</sup>. The energy of these compounds were optimized using MM2 force field methods<sup>[27]</sup> and converted to 3D format and saved as sybyl mol2 file format using ChemOffice 2010 (ChemOffice 2010: CambridgeSoft Corporation) for docking purposes.

### Cavity prediction

The cavity or the potential ligand binding site of Hsp90 (PDB ID: 3T0Z) was predicted using MVD. A cavity which has a volume of 151.04 Å<sup>3</sup> and a surface area of 462.08 Å<sup>2</sup> was predicted. The binding site was set inside a restriction sphere of 15 Å radius with the centre X: 12.67, Y: -3.46, Z: 14.09. The MolDock grid score was set with a grid resolution of 0.30 Å.

### Bond flexibility set up

The compounds viz. quercetin, kaempferol, myricetin and ATP molecule was loaded in the MVD. The bond flexibility of these compounds was set. Additionally, the side chain flexibility of the amino acid residues at the potential ligand binding site (Asn51, Ala55, Met98, Gly135, Val136, Gly137 and

Thr184) of Hsp90 was set with a tolerance of 1.10 and strength of 0.80 for docking simulations.

### Molecular docking simulation

Molecular docking simulation was performed using Molegro Virtual Docker (MVD) 6.01. The software is based on a differential evolution algorithm; the solution of the algorithm considers the sum of the intermolecular interaction energy between the ligand and the protein and the intramolecular interaction energy of the ligand. The docking energy scoring function was based on the modified piecewise linear potential (PLP) with new hydrogen bonding and electrostatic terms included<sup>[28]</sup>. The docking algorithm was set with softens potentials during the docking simulation with the side chains of the enzyme made flexible. The maximum minimization for the residues and the ligand was set at 2,000 steps and the maximum global minimization was set for 2,000 steps.

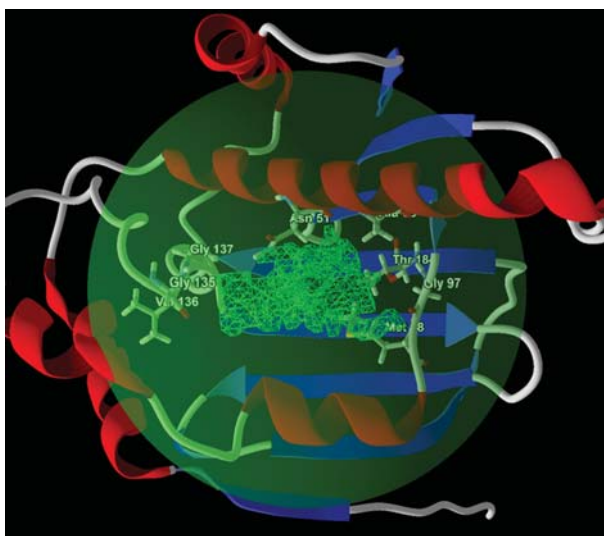
The MolDock scoring function was also set with a grid resolution of 0.30 Å. It was set at a maximum iteration of 1,500 with a simplex evolution size of 50 and a minimum of 10 runs were performed for each compound with threshold energy of 100. Additionally, the simplex evolution was set for 300 steps with a neighbour distance factor of 1.00. The best pose of each compound was selected for subsequent ligand-protein interaction energy analysis.

### Absorption, distribution, metabolism, excretion and toxicity (ADME-toxicity) analysis

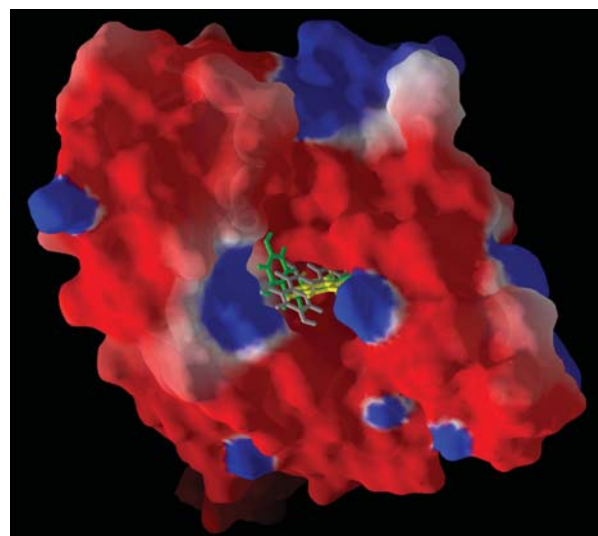
ADME-toxicity was carried out for quercetin, kaempferol and myricetin using ACD/I-Lab 2.0 (ACD/I-Lab, Version 2.0, Advanced Chemistry Development, Inc, Toronto, ON, Canada). The absorption, solubility, blood brain barrier (BBB) transport, oral bioavailability and distribution of quercetin, kaempferol and myricetin were calculated. Additionally, the LD<sub>50</sub> and probability of health effects of the 3 compounds were also calculated. Lastly, a comparative analysis was carried out of LD<sub>50</sub> in mouse (intraperitoneal, oral, intravenous and subcutaneous) for quercetin, kaempferol and myricetin.

## Results

Molecular docking simulation was carried out using the MVD. The binding cavity used in the present molecular docking simulation is shown in **Fig. 1**. The docking score and scoring results are shown in **Table 1**. The interaction energy of quercetin, kaempferol and myricetin is -109.87 kJ/mol, -104.73 kJ/mol and -99.48 kJ/mol, respectively, compared to -98.62



**Fig. 1** The potential ligand binding cavity of Hsp90 (PD ID: 3T0Z) with flexible residues predicted using MVD.



**Fig. 2** Surface map of HSp90 depicting quercetin (green), kaempferol (yellow) and myricetin (grey) lying deep into the binding pocket.

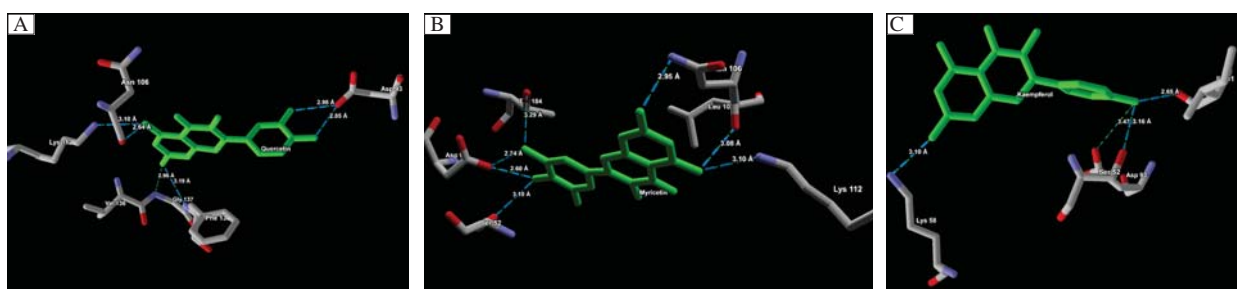
kJ/mol of ATP molecule. These findings indicated that quercetin, kaempferol and myricetin had more favorable ligand-protein interaction energy than ATP molecule at the binding cavity of Hsp90. In this study, the

molecular interaction of quercetin, kaempferol and myricetin lay deep inside the binding pocket of Hsp90, exhibiting both bonded and non-bonded interaction (**Fig. 2**).

**Table 1** Docking score of quercetin, kaempferol and myricetin and ATP molecule

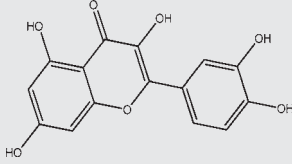
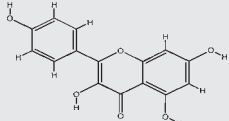
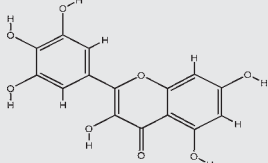
Name	Rerank score <sup>a</sup>	Interaction <sup>b</sup>	Internal <sup>c</sup>	HBond <sup>d</sup>	LE1 <sup>e</sup>	LE3 <sup>f</sup>
CID5280343 Quercetin	-71.27	-109.87	30.13	-12.49	-3.62	-3.24
CID5280863 Kaempferol	-64.87	-104.73	28.20	-7.85	-3.64	-3.09
CID5281672 Myricetin	-52.03	-99.48	39.71	-18.42	-2.60	-2.26
CID5957 ATP	61.21	-98.62	16.45	-4.94	-2.65	1.97

<sup>a</sup>The rerank score is a linear combination of E-inter (steric, Van der Waals, hydrogen bonding, electrostatic) between the ligand and the protein, and E-intra. (torsion, sp2-sp2, hydrogen bonding, Van der Waals, electrostatic) of the ligand weighted by pre-defined coefficients<sup>[28]</sup>. <sup>b</sup>The total interaction energy between the pose and the protein (kJ/mol). <sup>c</sup>The internal energy of the pose. <sup>d</sup>Hydrogen bonding energy (kJ/mol). <sup>e</sup>Ligand efficiency 1: MolDock score divided by heavy atoms count. <sup>f</sup>Ligand efficiency 3: Rerank score divided by heavy atoms count.



**Fig. 3** Molecular interaction of (A) quercetin (B) myricetin and (C) kaempferol at the active site of Hsp90. Green: steric interaction favorable; turquoise: hydrogen acceptor favorable; yellow: hydrogen donor favorable; red and blue: electrostatic potential of Hsp90.

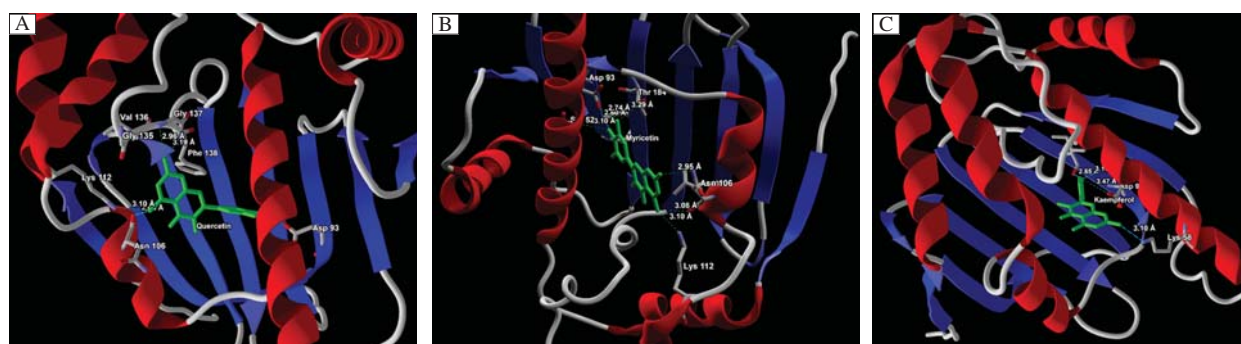
**Table 2** Molecular interaction analysis of quercetin, kaempferol and myricetin at the active site of Hsp90

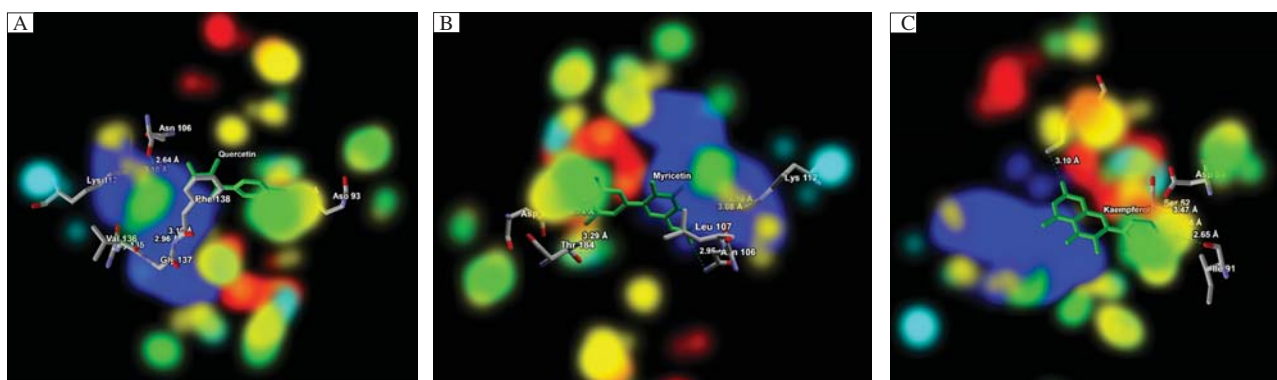
SN	Ligand	Protein-ligand interaction	Interaction distance	Interaction energy (kJ/mol)
1.		Asp93(OD2).....O(5)	2.98 Å	-2.5
		Asp93(OD2).....O(6)	2.85 Å	-2.5
		Asn106(O).....O(2)	2.64 Å	-2.5
		Lys112(NZ).....O(2)	3.10 Å	-2.5
		Phe138(N).....O(4)	3.19 Å	-2.01
		Gly137(N).....O(4)	2.96 Å	-0.45
2.		Ile91(O).....O(5)	2.65 Å	-2.5
		Asp93(OD2).....O(5)	3.47 Å	-0.65
		Ser52(OG).....O(5)	3.16 Å	-2.2
3.		Lys58(NZ).....O(4)	3.10 Å	-2.5
		Ser52(OG).....O(7)	3.10 Å	-2.48
		Asp93(OD2).....O(7)	2.60 Å	-2.5
		Asp93(OD2).....O(5)	2.74 Å	-2.5
		Thr184(OG1).....O(5)	3.29 Å	-1.53
		Asn106(ND2).....O(4)	2.95 Å	-2.5
		Asn106(O).....O(2)	3.08 Å	-2.5
		Lys112(NZ).....O(2)	3.10 Å	-2.5

Additionally, the molecular interaction analysis for the ligand-protein interaction is shown in **Table 2**. The average molecular interaction energy of quercetin is -2.07A kJ/mol, while that of kaempferol is -1.96 kJ/mol and -2.36 kJ/mol for myricetin. The snapshots of ligand-protein interaction depicting the binding mode of quercetin, kaempferol and myricetin are shown in **Fig. 3A, B** and **C**. Quercetin showed molecular interaction with Asp93, Asn106, Lys112, Gly137 and Phe138 residues of Hsp90 while kaempferol established molecular interaction with Ser52, Lys58, Ile91 and Asp93 residues of Hsp90 and myricetin had mole-

cular interaction with Ser52, Asp93, Asn106, Lys112 and Thr184 residues of Hsp90. The snapshots representing the secondary structures for the molecular interaction of quercetin, kaempferol and myricetin at the active site of Hsp90 are shown in **Fig. 4 A, B** and **C**, respectively. The top three docking hits showed common molecular interaction with Asp93 (OD2).

The energy map of Hsp90 that might contribute in steric interaction favourable (green colour), hydrogen acceptor favourable (turquoise colour), hydrogen donor favourable (yellow colour) and electrostatic potential of Hsp90 (red and blue colour) with the

**Fig. 4** Molecular binding mode of (A) quercetin (B) myricetin and (C) kaempferol at the binding site of Hsp90.



**Fig. 5** Energy map of (A) quercetin (B) myricetin and (C) kaempferol at the binding cavity of Hsp90.

ligand viz. quercetin, kaempferol and myricetin are shown in **Fig. 5 A, B** and **C**, respectively. The electrostatic interaction of quercetin, kaempferol and myricetin as well as the active site of Hsp90 are shown in **Fig. 6 A, B** and **C**, respectively.

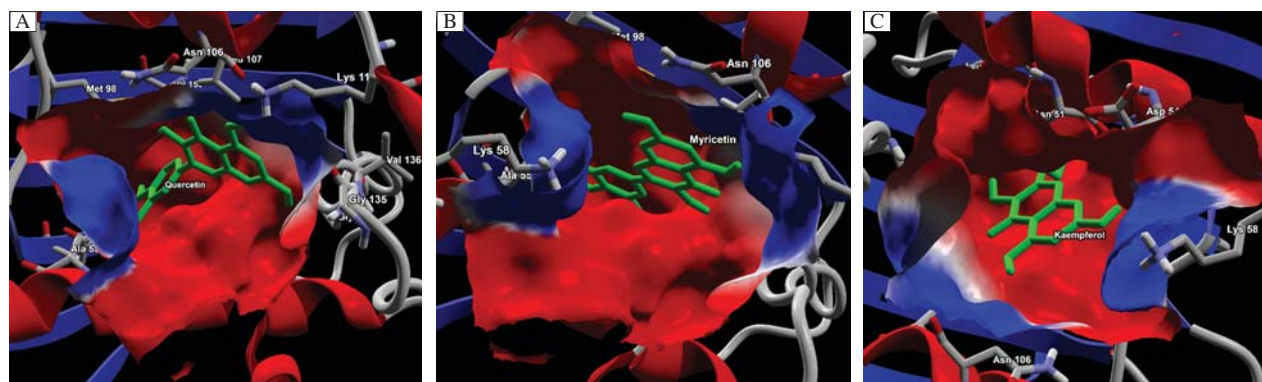
The results of the ADME-toxicity analysis calculated using ACD/Ilab 2.0 are shown in **Table 4** and the comparative graph plot on  $LD_{50}$  mouse for quercetin, kaempferol, and myricetin is shown in **Fig. 7**. ADME-toxicity analysis showed that kaempferol (0.10 mg/mL) was readily soluble compared to quercetin (0.14 mg/mL) and myricetin (0.25 mg/mL). For absorption, myricetin showed a bit lower passive absorption of 95% compared quercetin and kaempferol (100% passive absorption).

## DISCUSSION

It is revealed from the docking score and scoring result (**Table 1**) that quercetin, kaempferol and myricetin showed better rerank score than ATP molecule, indicating that the dietary flavanols docked at the active site of the Hsp90 (**Table 1**). The rerank score used in MVD is a weighted combination of the terms used by the

MolDock score mixed with a few addition terms which includes the *Steric* terms which are Lennard-Jones approximations to the steric energy<sup>[23]</sup>. The reranking score function is computationally more expensive than the scoring function used during the docking simulation, but it generally gives better result than the docking score function. The reranking coefficients used the energy parameters such as E-Inter total, E-Inter (protein-ligand), Steric, VdW (Van der Waal's), HBond (hydrogen bonding energy), E-Intra (tors, ligand atoms), E-Solvation, E-Total etc. In addition, as shown in **Fig. 2**, quercetin, kaempferol and myricetin were found to be lying deep inside the binding pocket of Hsp90 which is an indication of a strong molecular interaction exhibiting both bonded and non bonded interaction.

The average molecular interaction energy of the three compounds was also calculated which supports the binding affinity of these compounds at the binding site. Hsp90 is known for its high-affinity for binding the ATP molecule at a region near the N-terminus known as the ATP-binding region and when it is prevented from binding the ATP molecule, Hsp90 eventually is degraded. Hence, these dietary flavanols will



**Fig. 6** Electrostatic interaction (A) quercetin (B) myricetin and (C) kaempferol at the binding cavity of Hsp90.

**Table 3 Lipinski rule of 5 parameters for quercetin, myricetin, kaempferol and ATP molecule**

SN	Compound	HBD	HBA	Mol. Wt	XLog P3	Rot B	TPSA
1	Quercetin	5	7	302.23	1.5	1	127
2	Kaempferol	4	6	286.24	1.9	1	107
3	Myricetin	6	8	318.24	1.2	1	148
4	ATP	7	17	507.18	-5.7	8	279

HDB: hydrogen bond donor; HBA: hydrogen bond acceptor; Mol. Wt.: molecular weight; Rot B: rotatable bonds; TPSA: topological polar surface area.

serve as a good inhibitor of Hsp90 showing competitive inhibition with ATP molecule as evident from the docking score. Moreover the Lipinski rule of five parameters for quercetin, kaempferol, myricetin and ATP indicates that these dietary flavonols do not violate the rule of five to be an orally active compound inside the human body.

The comparative graph plot on LD<sub>50 mouse</sub> for quercetin, kaempferol, and myricetin indicated that quercetin, kaempferol, and myricetin had higher LD<sub>50 mouse</sub> (oral). Hence, it can be administered orally instead of intravenously or subcutaneously. In fact, the phytochemicals possessed enhanced pharmacological prop-

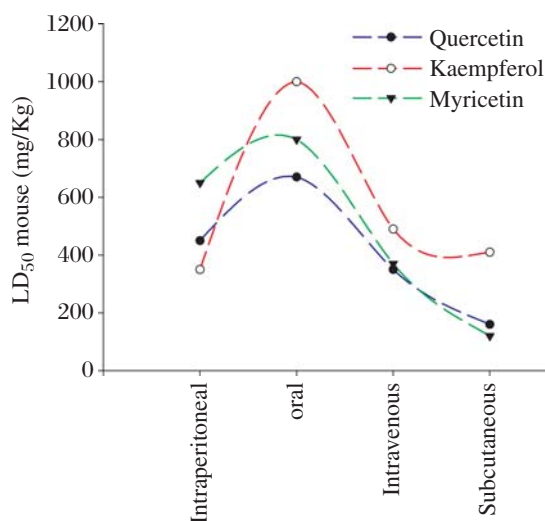
erties with lesser health effects. Thus, these dietary phytochemicals may serve as lead molecule or a potent inhibitor of Hsp90.

In this study, the authors have carried out molecular docking simulation and molecular interaction analysis of 3 dietary flavonols against Hsp90. The molecular docking score revealed that the dietary phytochemicals viz. quercetin, myricetin and kaempferol showed competitive inhibition with ATP molecule at the high affinity ATP binding site. They inhibited the ATP binding pocket which will lead to the degradation of Hsp90. Furthermore, the inhibition of Hsp90 enzyme will contribute to the decreasing for stabilization of several pro-

**Table 4 Predicted ADME-toxicity parameters for quercetin, kaempferol and myricetin**

ADME-toxicity parameters	CID5280343 Quercetin	CID5280863 Kaempferol	CID5281672 Myricetin
Solubility <sup>a</sup> H <sub>2</sub> O (mg/mL)	0.14	0.10	0.25
BBB <sup>b</sup> Log PS	-2.8	-2.4	3.4
BBB <sup>b</sup> Log PB	-0.73	-0.65	-0.75
BBB <sup>b</sup> Log(PS*fu, brain)	-3.3	-2.9	-3.7
% Oral bioavailability <sup>c</sup>	>30	>30	>30
Absorption <sup>d</sup> (% Passive absorption)	100	100	95
Absorption <sup>d</sup> (Permeability, cm/second)	$3.37 \times 10^{-4}$	$5.85 \times 10^{-4}$	$0.97 \times 10^{-4}$
Distribution <sup>e</sup> (L/kg) <sup>e</sup>	0.6	0.61	0.59
LD <sub>50mouse</sub> <sup>f</sup> (mg/kg, intraperitoneal)	450	350	650
LD <sub>50mouse</sub> <sup>f</sup> (mg/kg, oral)	670	1000	800
LD <sub>50mouse</sub> <sup>f</sup> (mg/kg, intravenous)	350	490	370
LD <sub>50mouse</sub> <sup>f</sup> (mg/kg, subcutaneous)	160	410	120
Prob. of blood effect <sup>g</sup>	0.34	0.3	0.86
Prob. of cardiovascular system effect <sup>g</sup>	0.8	0.76	0.62
Prob. of gastrointestinal system effect <sup>g</sup>	0.72	0.68	0.89
Prob. of kidney effect <sup>g</sup>	0.79	0.52	0.79
Prob. of liver effect <sup>g</sup>	0.3	0.27	0.3
Prob. of lung effect <sup>g</sup>	0.38	0.31	0.8

<sup>a</sup>Calculates compound's solubility in a buffer at a specified pH value. <sup>b</sup>Calculates the Blood Brain Barrier (BBB) transport (LogPS: rate of brain penetration; LogPB: extent of brain penetration; Log (PS\*fu, brain): brain/plasma equilibration rate). <sup>c</sup>Estimates the probability of a compounds bioavailability being above 30% and 70%. <sup>d</sup>Estimates maximum passive absorption and human jejunum permeability. <sup>e</sup>Calculates the apparent volume of distribution for a compound in L/kg. <sup>f</sup>Estimates LD<sub>50</sub> value in mg/kg after intraperitoneal, oral, intravenous and subcutaneous administration to mice. <sup>g</sup>Estimates probability of blood, gastrointestinal system, kidney, liver and lung effect at therapeutic dose range.



**Fig. 7** LD<sub>50</sub> mouse (intraperitoneal, oral, intravenous, subcutaneous) graph plot for quercetin, kaempferol and myricetin.

teins which are involved in tumour growth and cancer. Moreover, there are only preliminary reports of these flavonols reporting for anti-cancer property or inhibiting Hsp90. Additionally, the dietary phytochemicals used in the present study do not violate the Lipinski rule of 5 parameters.

In addition, from the ADME-toxicity analysis, these compounds possessed enhanced pharmacological parameters with lesser LD<sub>50</sub> and health effects. Hence, we conclude that, quercetin, myricetin and kaempferol will contribute to fight against cancer thereby inhibit the chaperonic function of Hsp90, which support experimental testing of these compounds.

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