Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary Information

Molecular recognition of bio-active flavonoids quercetin and rutin by bovine hemoglobin: An overview of the binding mechanism, thermodynamics and structural aspects through multi-spectroscopic and molecular dynamics simulation studies

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Figure S1

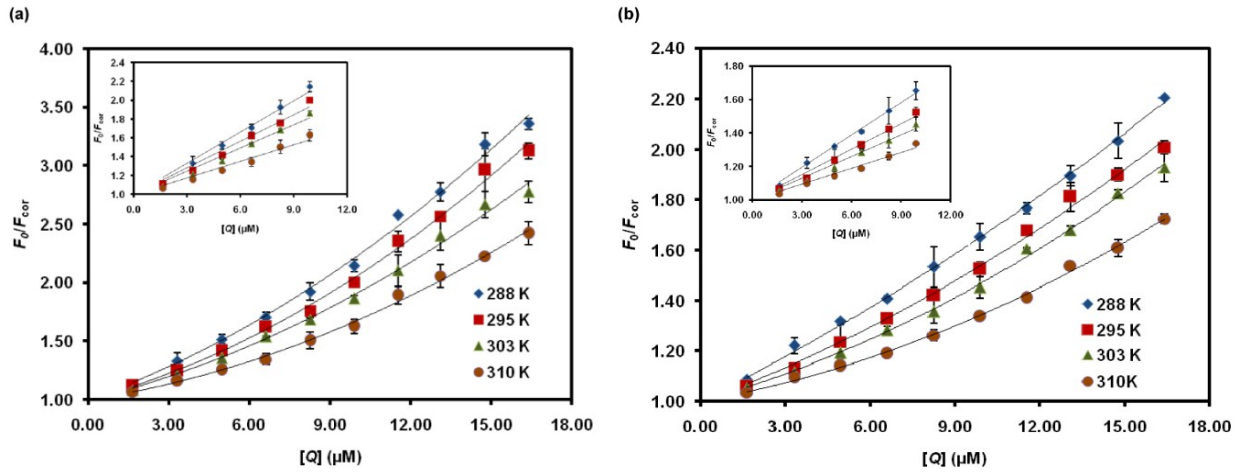


Figure S1: Stern Volmer plots for the interaction of (a) quercetin and (b) rutin with BHb at different temperatures. ***Inset*** represents the corresponding linear range plot. [BHb]= 3μM, [Flavonoids]= 0-16.4 μM, λ_{ex}= 295 nm.

Figure S2

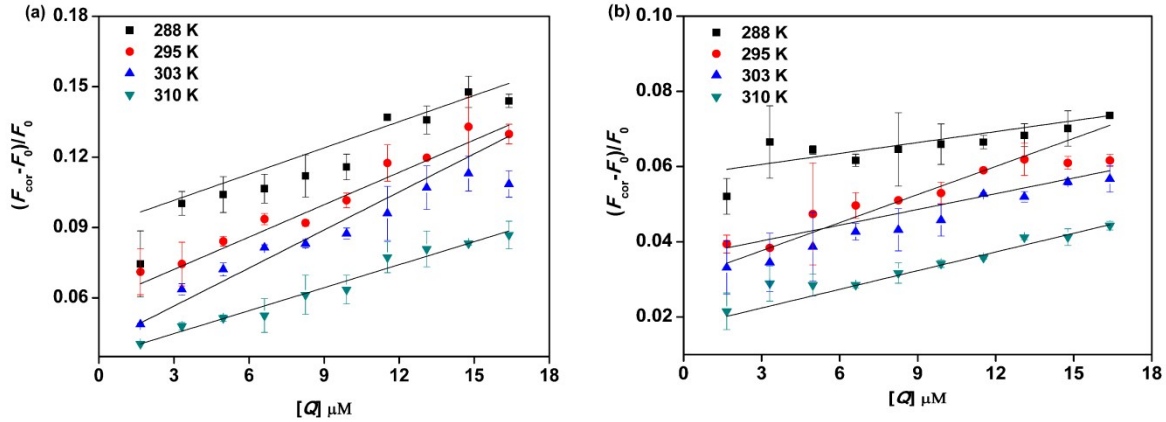


Figure S2: Regression plot for the interaction of (a) quercetin and (b) rutin with BHb. λ_{ex}=295 nm.

Figure S3

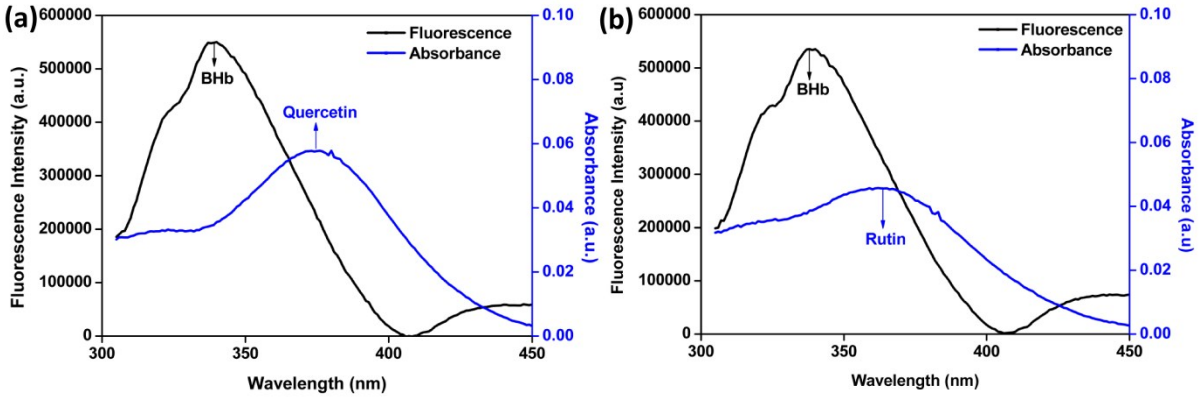


Figure S3: Spectral overlap of the fluorescence emission spectra of BHb (black line) and the absorption spectrum of (a) quercetin and (b) rutin (blue lines) in 20 mM PB of pH 7.4. [BHb]=[Flavonoids]= 3 μM, λ_{ex}= 295 nm.

Figure S4

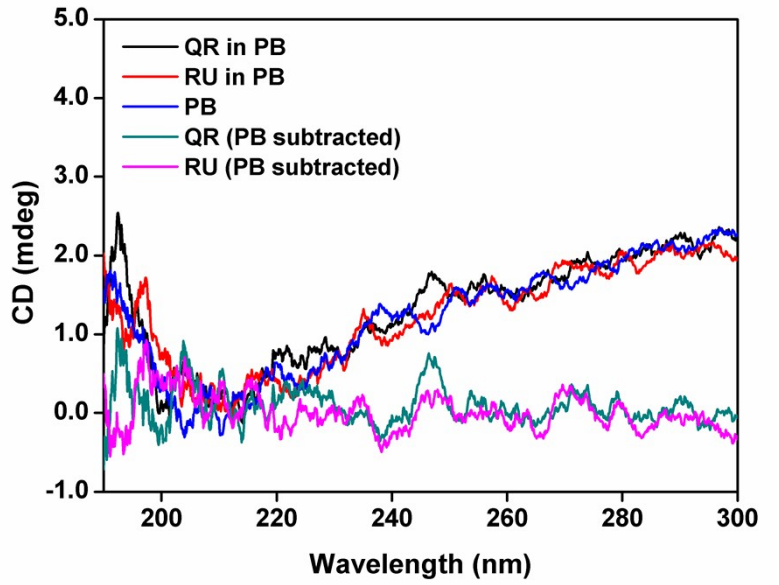


Figure S4: CD spectra of 20 mM PB (pH 7.4), quercetin (QR), rutin (RU), and the difference spectra of QR and Rutin. PB= Phosphate buffer, [QR]=[RU]= 6 μM. (Instrument: JASCO J-1500).

Figure S5

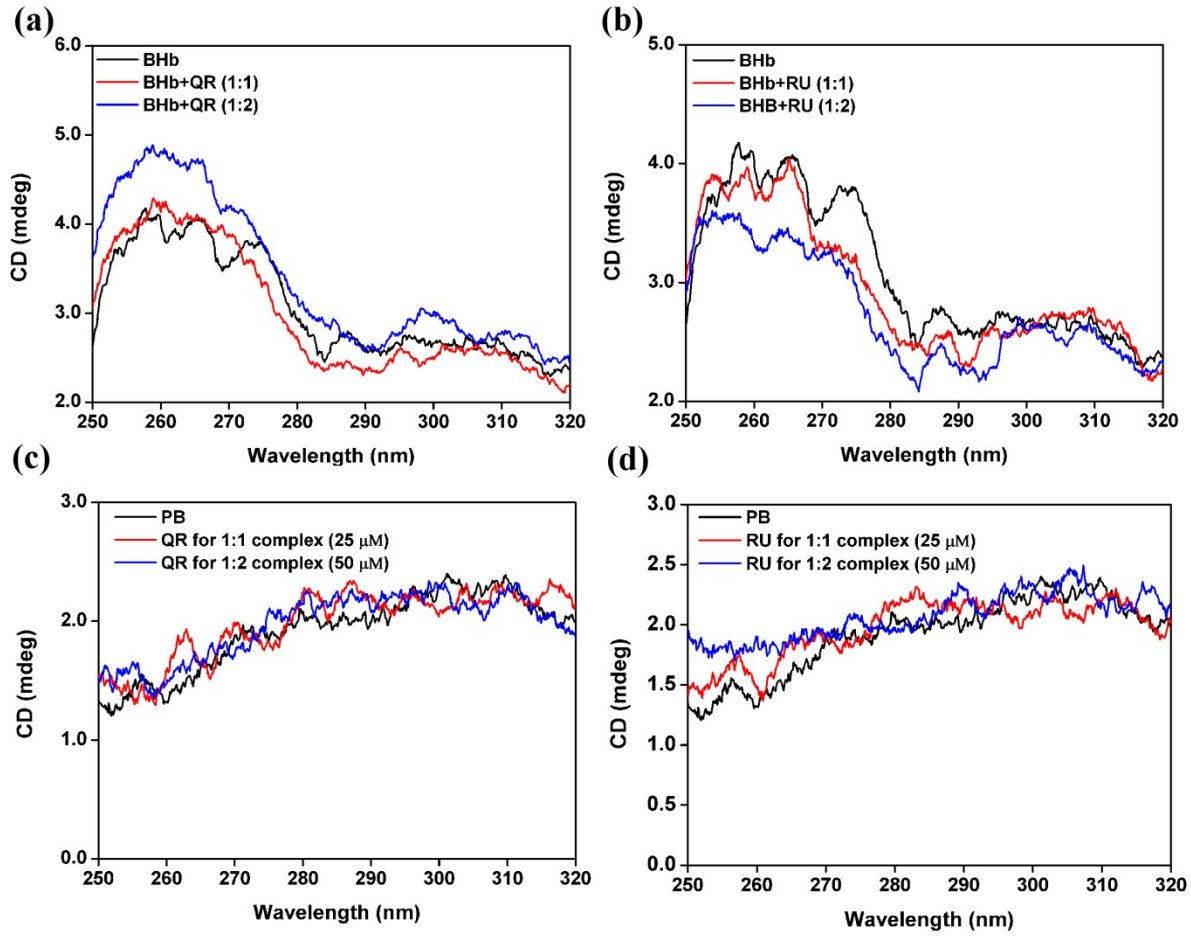


Figure S5: Near-UV CD spectra of 25 μM BHb in the presence of (a) quercetin (QR) and (b) rutin (RU) at a molar ratio of 1:1 and 1:2. Panels (c) and (d) denote the blank spectra of phosphate buffer (PB), QR and RU.

Figure S6

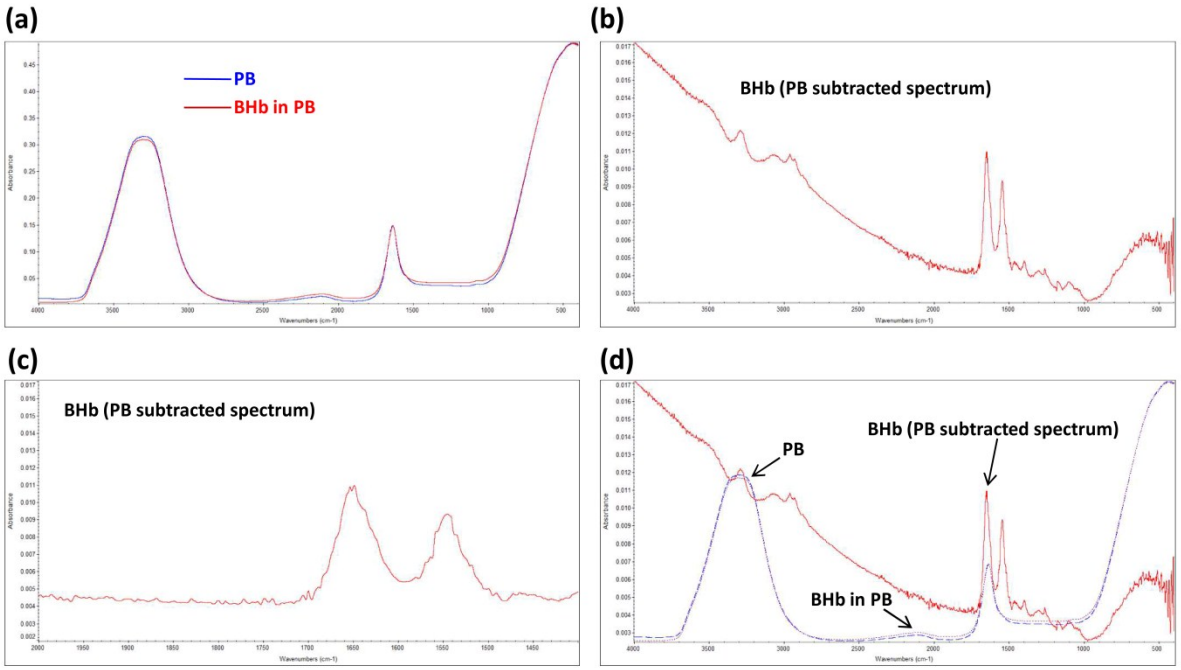


Figure S6: FT-IR spectra of (a) phosphate buffer (PB) and BHb in 20 mM PB of pH 7.4. (b) BHb after subtracting the PB spectrum in the region of 4000-400 cm^{-1}. (c) BHb spectra in the expanded region of 1800-1400 cm^{-1}. (d) Merged spectra of the native BHb, PB and subtracted BHb.

Figure S7

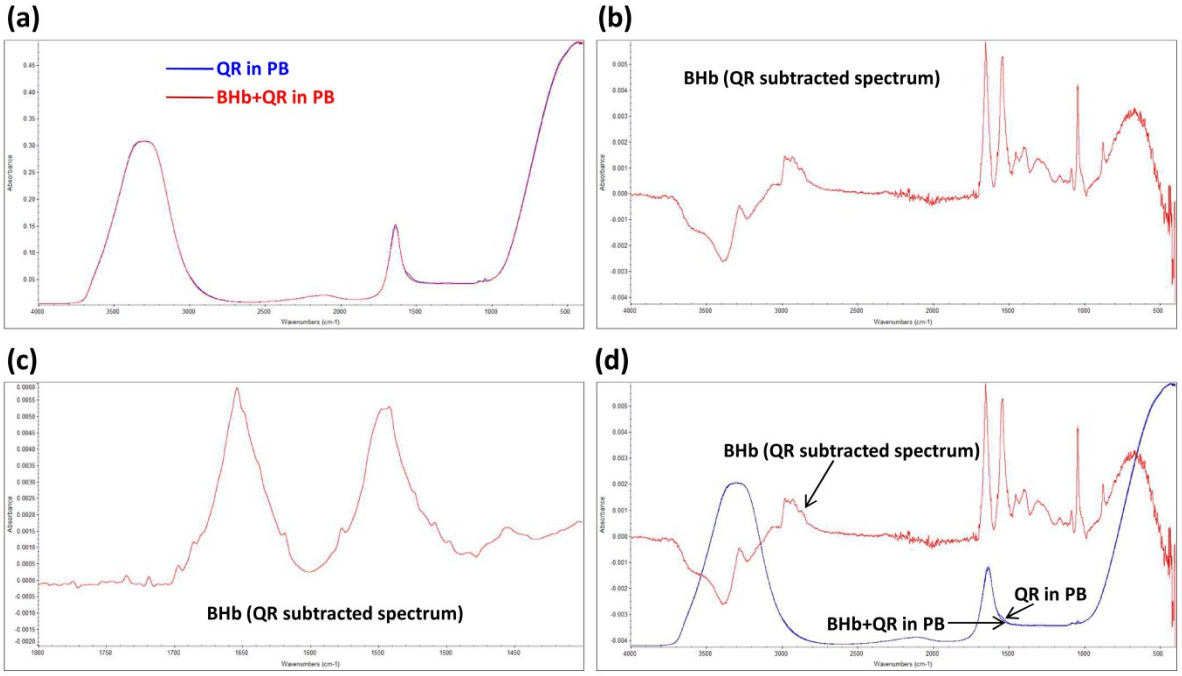


Figure S7: FT-IR spectra of (a) quercetin (QR) and its complex with BHb in 20 mM PB of pH 7.4. (b) BHb after subtracting the QR spectrum in the region of 4000-400 cm^{-1}. (c) BHb spectra in the expanded region of 1800-1400 cm^{-1}. (d) Merged spectra of the native BHb, native QR and subtracted BHb.

Figure S8

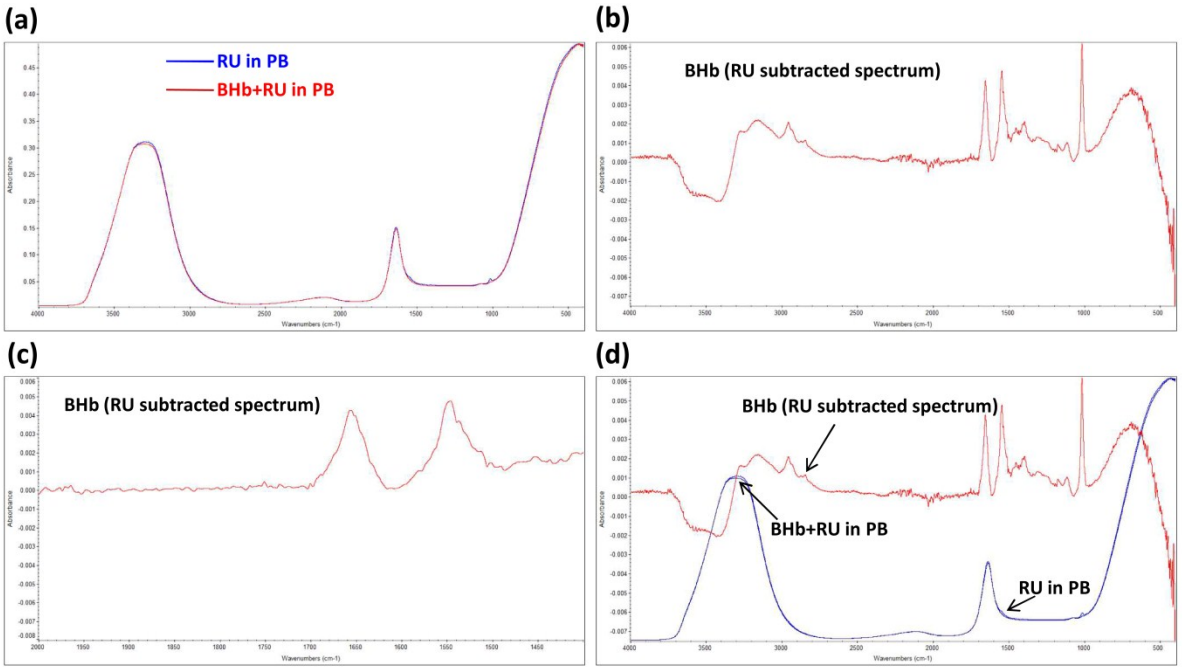
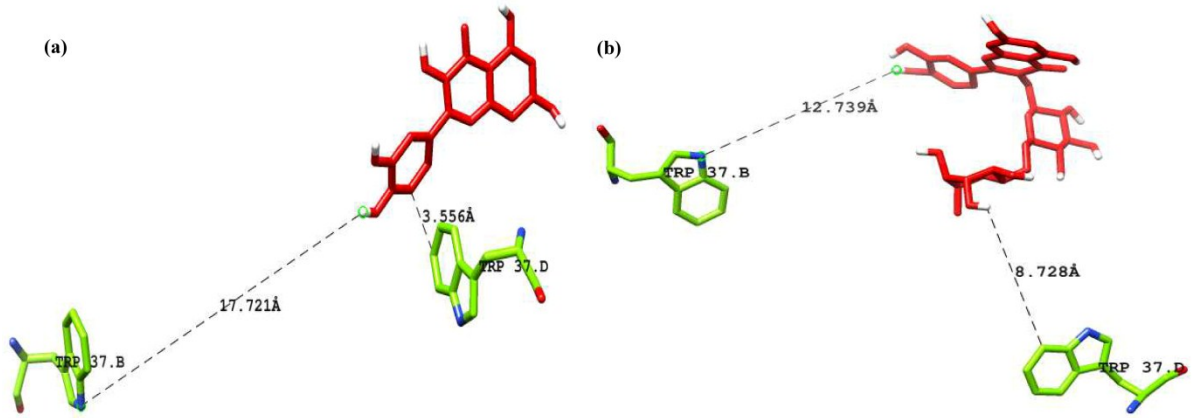


Figure S8: FT-IR spectra of (a) rutin (RU) and its complex with BHb in 20 mM PB of pH 7.4. (b) BHb after subtracting the RU spectrum in the region of 4000-400 cm^{-1}. (c) BHb spectra in the expanded region of 1800-1400 cm^{-1}. (d) Merged spectra of the native BHb, native RU and subtracted BHb.

Figure S9



*B= β_1 chain, D= β_2 chain

Figure S9: The distance of (a) quercetin and (b) rutin from β_1-Trp37 and β_2-Trp37 residues of BHb as predicted by the molecular docking studies.

Figure S10

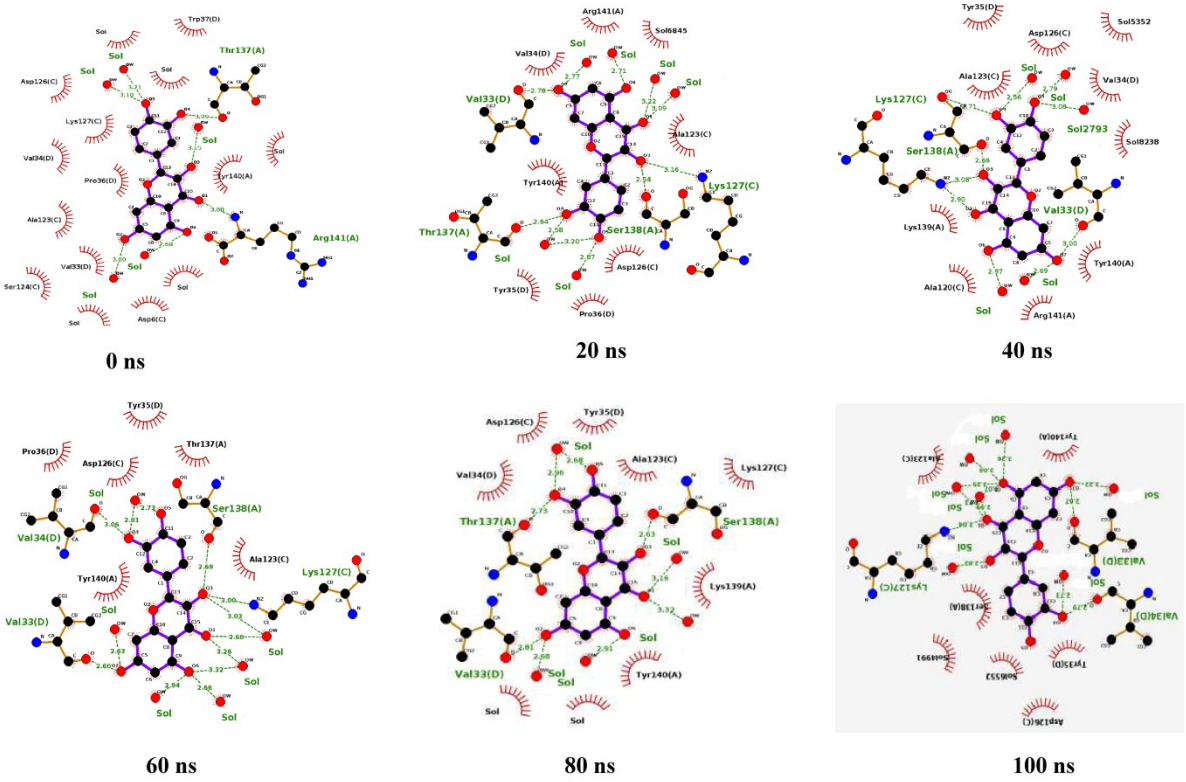


Figure S10: The 2D interaction maps of quercetin with different amino residues of BHb observed during 100 ns MD simulation period.

Figure S11

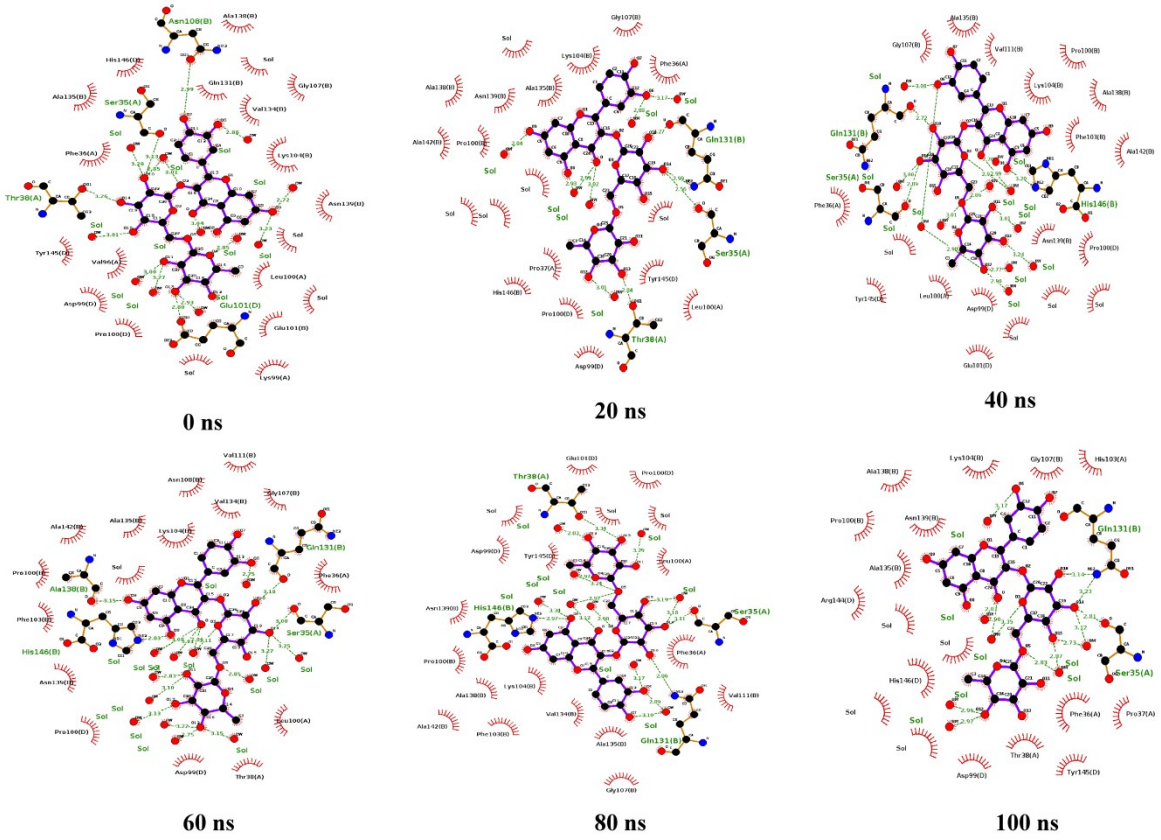
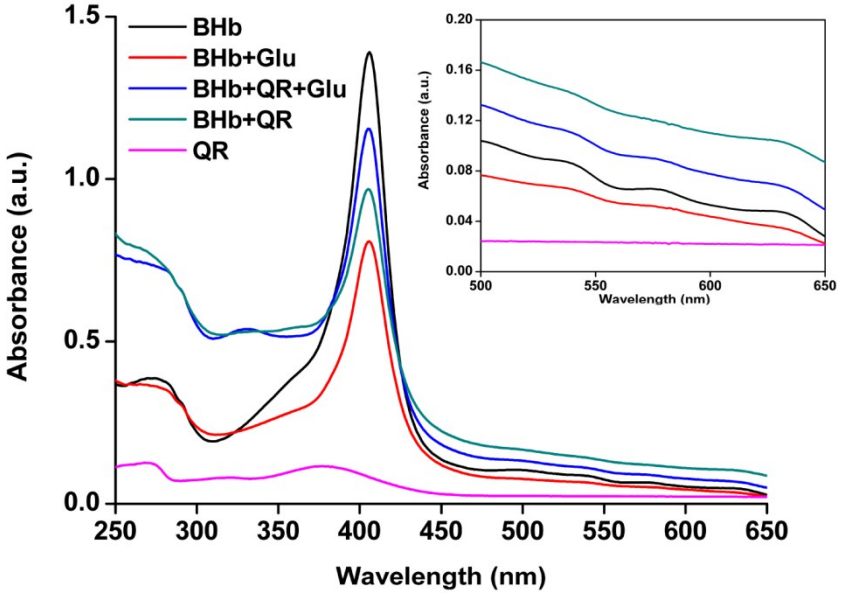


Figure S11: The 2D interaction maps of rutin with different amino residues of BHb observed during 100 ns MD simulation period.

Figure S12



*QR=Quercetin, Glu= Glucose.

Figure S12: The absorption spectra of native BHb and its complex in the presence of glucose and quercetin in 20 mM PB of pH 7.4 at 37°C. ***Inset*** shows the corresponding spectrum in the region of 500-650 nm. The samples were incubated for 72 h.

Figure S13

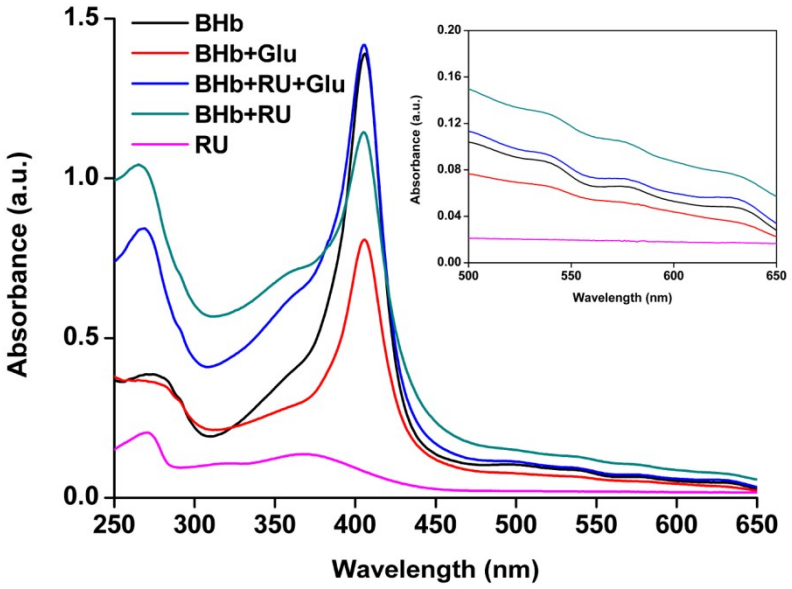


Figure S13: The absorption spectra of native BHb and its complex in the presence of glucose and rutin in 20 mM PB of pH 7.4 at 37°C. ***Inset*** shows the corresponding spectrum in the region of 500-650 nm. The samples were incubated for 72 h.

Table S1: Stern Volmer parameters for the interactions of quercetin and rutin with BHb at different temperatures.

Ligands	**Temp. (K)**	K_{SV} **(10^4,M^{-1})**	k_q **(10^{13}, $M^{-1}s^{-1}$)**
Quercetin	288	10.53±0.46	6.46
	295	9.10±0.32	5.58
	303	7.93±0.27	4.86
	310	6.05±0.29	3.71
Rutin	288	6.33±0.12	3.88
	295	5.04±0.23	3.09
	303	4.36±0.16	2.67
	310	3.16±0.32	1.93

Table S2: The 3D spectral characteristics of BHb and its 1:1 complexes with the flavonoids.

System	Peak	Peak position ($\lambda_{em}/\lambda_{ex}$) (nm/nm)	Stokes shift $\Delta\lambda$(nm)	Intensity (a.u.)
BHb	1	285/337	52	84.443
	2	235/337	102	61.034
BHb-RU	1	285/339	54	74.449
	2	235/337	102	50.503
BHb-QR	1	285/339	54	73.431
	2	235/337	102	49.860

* QR: Quercetin, RU: Rutin.

Table S3: Docking summary of BHb with quercetin by Autodock 4.2 program generating different ligand conformations with the help of Lamarckian GA.

Rank	Binding Energy (kcal mol^{-1})	K_i (μM)	K_b (10^4, M^{-1})	Cluster rmsd	Reference rmsd
1	-7.80	4.61	50.60	0.00	69.20
2	-7.17	5.56	17.51	1.21	68.35
3	-6.78	10.76	9.08	1.95	67.82
4	-6.81	10.17	9.55	0.00	72.14
5	-6.27	25.3	3.84	0.00	70.09
6	-6.03	37.97	2.56	0.00	67.37
7	-6.02	38.66	2.52	0.02	67.37
8	-5.95	43.81	2.24	0.00	68.99
9	-5.86	50.37	1.92	0.00	47.86
10	-5.63	74.62	1.31	0.00	61.76

Table S4: Docking summary of BHb with rutin by Autodock 4.2 program generating different ligand conformations with the help of Lamarckian GA.

Rank	Binding Energy (kcal mol^{-1})	K_i (μM)	K_b (10^4, M^{-1})	Cluster rmsd	Reference rmsd
1	-6.92	8.44	11.49	0.00	50.75
2	-6.62	14.08	6.93	0.00	63.72
3	-6.6	14.61	6.70	0.00	64.99
4	-5.71	65.08	1.49	0.00	48.82
5	-4.87	269.26	0.36	0.00	54.09
6	-4.15	914.89	0.10	0.00	51.73
7	-4.06	1140	0.09	0.00	66.02
8	-3.59	2350	0.04	0.00	52.92
9	-3.31	3740	0.02	0.00	65.43
10	-2.47	15490	0.01	0.00	52.08

Table S5: Accessible surface area of different chains in BHb upon interaction with the flavonoids.

System	α_1	β_1	α_2	β_2	Total
BHb	5771.2	6128.1	5858.0	6047.4	23804.6
BHb-Quercetin	5687.2	6128.1	5762.6	5990.6	23652.5
BHb-Rutin	5652.0	5930.9	5858.0	5938.9	23488.1

Table S6: Hydrogen bonds formed by the flavonoids with the amino acid residues of BHb during the simulation period along with the number of hydrophobic interactions

Ligands	Time	No. of H-Bonds	Residues Involved	No of Hydrophobic Interactions
Quercetin	Docked Complex	5	α_1-Thr137, β_2-Val33, α_2-Lys127, α_1-Arg141, α_1-Ser 138	6
	0 ns	2	α_1-Thr137, α_1-Arg141	15
	20 ns	4	α_1-Thr137, β_2-Val33, α_1-Ser138, α_2-Lys127	8
	40 ns	5	β_2-Val33, α_1-Ser138 (2), α_2-Lys127 (2)	10
	60 ns	4	β_2-Val33, β_2-Val34, α_1-Ser138, α_2-Lys127	6
	80 ns	3	β_2-Val33, α_1-Thr137, α_1- Ser138	9
	100 ns	3	β_2-Val33, β_2-Val34, α_2-Lys127	7
Rutin	Docked Complex	5	β_1-Lys104 (2), β_2-Glu101, β_2-Asp 99 (2)	14
	0 ns	4	α_1-Ser35, α_1-Thr38, β_2-Glu101, β_1-Asn 108	20
	20 ns	4	β_1-Gln131 (2), α_1-Ser35, α_1-Thr38	19
	40 ns	3	β_1-His146, β_1-Gln131, α_1-Ser35	19
	60 ns	4	β_1-Gln131, α_1-Ser35, β_1-His146, β_1-Ala138	16
	80 ns	4	β_1-Gln131, β_1-His146, α_1-Ser35, α_1-Thr38	19
	100 ns	3	β_1-Gln131 (2), α_1-Ser35	18

***** The number within parentheses ( ) represents the number of hydrogen bonds formed with that amino acid residue.