In silico, In Vitro and *In Vivo* Evaluation of the developmental toxicity, estrogenic activity and mutagenicity of four natural phenolic flavonoids at low exposure levels

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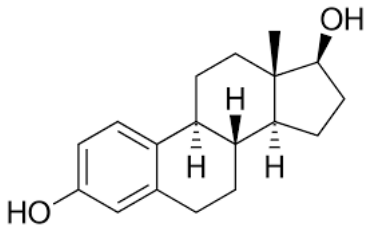
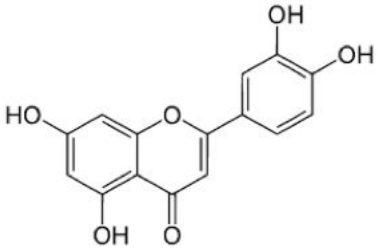
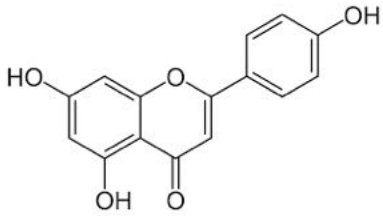
Supplemental materials (Tables and Figures)

Table S1. Binding energy of E2, luteolin, apigenin, quercetin, and genistein for 14 nuclear receptors with 4 antagonist conformation. There are four probability binding classes: red (high level of binding), orange (good binding), yellow (medium binding), and green (low binding).

Ligand	Structure	Binding energy with receptors (kcal/mol)		
E2		AR : -10.5	AR an.: -10.1	ER α: -10.6
		ER α an.: -10.7	ER β: -10.0	ER β an.: -9.2
		GR: -9.5	GR an.: -8.0	LXR α: -10.4
		LXR β: -10.8	MR: -8.6	PPAR α: -8.0
		PPAR β: -8.2	PPAR γ: -8.0	PR: -2.7
		RXR α: -8.8	TR α: -9.6	TR β: -9.8
Luteolin		AR : -9.1	AR an.: -9.0	ER α: -8.6
		ER α an.: -9.3	ER β: -7.6	ER β an.: -8.6
		GR: -9.2	GR an.: -8.0	LXR α: -9.0
		LXR β: -9.6	MR: -9.3	PPAR α: -9.0
		PPAR β: -8.5	PPAR γ: -8.1	PR: -2.6
		RXR α: -9.7	TR α: -9.4	TR β: -9.5
Apigenin		AR : -8.9	AR an.: -9.1	ER α: -8.8
		ER α an.: -9.1	ER β: -8.2	ER β an.: -8.9
		GR: -8.8	GR an.: -7.8	LXR α: -8.8
		LXR β: -9.5	MR: -9.0	PPAR α: -9.3
		PPAR β: -8.2	PPAR γ: -9.3	PR: -2.5
		RXR α: -9.7	TR α: -9.6	TR β: -9.4

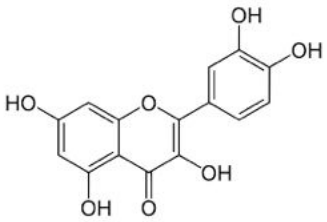
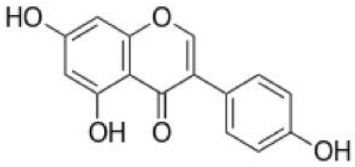
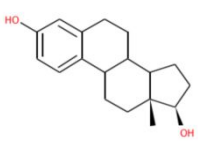
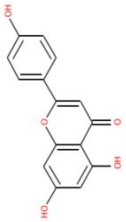
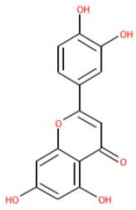
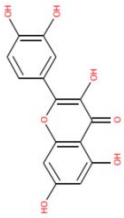
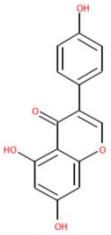
Quercetin		AR : -8.9 | AR an.: -8.6 | ER α: -8.2 ER α an.: -8.3 | ER β: -7.1 | ER β an.: -8.2 GR: -9.5 | GR an.: -8.1 | LXR α: -9.2 LXR β: -9.2 | MR: -9.1 | PPAR α: -7.7 PPAR β: -8.5 | PPAR γ: -9.3 | PR: -2.7 RXR α: -9.0 | TR α: -9.1 | TR β: -9.2
Genistein		AR : -9.1 | AR an.: -9.1 | ER α: -9.2 ER α an.: -9.2 | ER β: -8.7 | ER β an.: -8.7 GR: -8.9 | GR an.: -7.5 | LXR α: -8.7 LXR β: -9.6 | MR: -9.2 | PPAR α: -8.1 PPAR β: -8.6 | PPAR γ: -8.2 | PR: -2.4 RXR α: -8.9 | TR α: -9.6 | TR β: -9.6

Table S2. Acute toxicity, developmental toxicity, and mutagenicity of E2, luteolin, apigenin, quercetin, and genistein simulated by Toxicity Estimation Software Tool (T.E.S.T.). Photographs are from T.E.S.T. software.

Chemicals		E2	Apigenin	Luteolin	Quercetin	Genistein
Structure						
Fathead minnow LC50 (96 hr)	Fathead minnow LC50 (96 hr) - Log10(mol/L)	5.62	5.49	5.55	5.49	5.61
	Fathead minnow LC50 (96 hr) mg/L	0.65	0.88	0.81	0.97	0.66
Daphnia magna LC50 (48 hr)	Daphnia magna LC50 (48 hr) - Log10(mol/L)	4.91	5.06	4.8	4.54	4.62
	Daphnia magna LC_{50} (48 hr) mg/L	3.39	2.37	4.51	8.76	6.43
T. pyriformis IGC50 (48 hr)	T. pyriformis IGC50 (48 hr) - Log10(mol/L)	4.99	4.6	4.28	3.84	4.08
	T. pyriformis IGC50 (48 hr) mg/L	2.79	6.72	14.95	43.55	22.71
Oral rat LD50	Oral rat LD50 - Log10 (mol/kg)	2.36	2.2	2.12	2.04	2.36
	Oral rat LD50 mg/kg	1186.18	1707.99	2175.63	2782.81	1172.86

Bioaccumulation factor	Bioaccumulation factor Log10	2.19	1.31	1.19	0.58	1.21
	Bioaccumulation factor	153.42	20.62	15.46	3.84	16.21
Developmental Toxicity	Developmental Toxicity value	0.95	0.65	0.88	0.77	0.76
	Developmental Toxicity result	Developmental toxicant	Developmental toxicant	Developmental toxicant	Developmental toxicant	Developmental toxicant
Mutagenicity	Mutagenicity value	0.23	0.29	0.53	0.55	0.23
	Mutagenicity result	Mutagenicity Negative	Mutagenicity Negative	Mutagenicity Positive	Mutagenicity Positive	Mutagenicity Negative
Acute toxicity chemical classification		Class 4	Class 4	Class 5	Class 5	Class 4

Figure S1. Different types of malformation found in chicken embryo (A: normal; B: deformed claw observed in quercetin 500 µg/kg; C: stunting observed in luteolin 47.7 µg/kg treatment group).