













## Supplementary data to:

### Original article:

## ANTIVIRAL ACTIVITY OF MYRICETIN GLYCOSYLATED COMPOUNDS ISOLATED FROM *MAR CETIA TAXIFOLIA* AGAINST CHIKUNGUNYA VIRUS

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Raw data concerning **Figure 1**: Vero and BHK-21 cell lines were inoculated with CHIKV at an MOI of  $10^{-4}$ . Culture supernatants and infected cells were collected at 0, 10, 22, 34, 46, 70, and 106 hours. (1) Plaques were counted in plates previously seeded with 70.000 cells/mL incubated with the collected medium for five days. (2) RNA from infected cells was extracted, and the nsP1 CHIKV gene was amplified by RT-qPCR.

**Table 1:** Sample datasets were used to calculate the Chikungunya infectious titer by the Reed–Muench formula

Dilution		10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>	10 <sup>-8</sup>	10 <sup>-9</sup>	10 <sup>-10</sup>	Mock	
Scored cytopathic effect (CPE)	A	CPE	CPE	CPE	CPE	CPE	NON CPE	NON CPE	NON CPE	NON CPE	NON CPE	NON CPE	NON CPE
	B	CPE	CPE	CPE	CPE	CPE	CPE	NON CPE	NON CPE	NON CPE	NON CPE	NON CPE	NON CPE
	C	CPE	CPE	CPE	CPE	CPE	CPE	NON CPE	NON CPE	NON CPE	NON CPE	NON CPE	NON CPE
	D	CPE	CPE	CPE	CPE	CPE	CPE	NON CPE	NON CPE	NON CPE	NON CPE	NON CPE	NON CPE
	E	CPE	CPE	CPE	CPE	CPE	CPE	NON CPE	NON CPE	NON CPE	NON CPE	NON CPE	NON CPE
	F	CPE	CPE	CPE	CPE	CPE	CPE	NON CPE	NON CPE	NON CPE	NON CPE	NON CPE	NON CPE
	G	CPE	CPE	CPE	CPE	CPE	CPE	NON CPE	NON CPE	NON CPE	NON CPE	NON CPE	NON CPE
	H	CPE	CPE	CPE	CPE	CPE	CPE	NON CPE	NON CPE	NON CPE	NON CPE	NON CPE	NON CPE

**Table 2:** Ct values were obtained from RNA isolated from Vero and BHK-21 cell lines infected with CHIKV at MOI of  $10^{-4}$  at 0, 10, 22, 34, 46, 70, and 106 hours

Time	Vero				BHK-21			
	Ct <sub>1</sub>	Ct <sub>2</sub>	Ct <sub>3</sub>	Ct <sub>4</sub>	Ct <sub>1</sub>	Ct <sub>2</sub>	Ct <sub>3</sub>	Ct <sub>4</sub>
0	UD*	UD	UD	UD	UD	UD	UD	UD
10	36.448	36.363	36.326	36.528	32.209	32.581	32.317	32.992
22	33.832	33.431	33.990	33.359	30.261	30.494	30.232	30.297
34	28.225	29.559	28.268	28.431	25.631	25.691	25.929	25.450
46	24.711	24.081	24.734	24.297	22.011	22.651	22.647	22.732
70	22.347	22.980	22.253	22.834	20.935	20.448	20.284	20.690
106	21.811	21.800	21.945	21.830	20.864	20.617	20.597	20.270

\* UD: undetermined

Raw data concerning **Table 1**: Cytotoxic activity of *Marcetia taxifolia* flavonoids on BHK-21 cell line

**Table 3**: Absorbance values obtained by an MTT assay in BHK-21 cells infected with CHIKV and incubated with myricetin rhamnoside (MR), 5-hydroxy-3,6,7,8,3',4'-hexamethoxyflavona (HMF), 5,3'-dihydroxy-3,6,7,8,4'-pentamethoxyflavona (PMF) or myricetin 3-(6-rhamnosilgalactoside) (MRG) during 24, 48 and 72 hours

<b>MR</b>											
Concentration ( $\mu\text{g/mL}$ )											
	250	125	62.5	31.3	15.6	7.81	3.9	1.95	0.97	0.48	0
24 h	1.06	1.04	0.96	1.05	1.09	1.12	0.88	0.97	1.02	1.05	0.89
	1.01	1.13	1.22	0.86	0.98	0.78	0.81	0.93	0.96	0.94	0.83
	1.16	1.16	1.11	0.98	0.96	0.98	0.83	0.84	1.01	0.91	0.91
48 h	0.97	1.36	1.49	1.40	1.45	1.53	1.56	1.55	1.48	1.50	1.52
	0.87	1.41	1.31	1.29	1.36	1.50	1.38	1.44	1.47	1.27	1.47
	0.94	1.33	1.37	1.27	1.44	1.45	1.39	1.36	1.53	1.38	1.48
72 h	1.87	1.77	2.11	2.24	2.23	2.12	2.33	2.33	2.32	2.25	2.32
	1.74	1.66	1.87	1.88	2.18	1.97	1.93	2.12	2.21	2.13	2.03
	1.52	1.67	1.99	1.80	2.27	1.92	2.04	1.85	2.24	2.07	2.08
<b>HMF</b>											
Concentration ( $\mu\text{g/mL}$ )											
	250	125	62.5	31.3	15.6	7.81	3.9	1.95	0.97	0.48	0
24 h	0.11	0.11	0.77	0.64	0.50	0.41	0.55	0.67	0.81	0.72	0.73
	0.11	0.11	0.95	0.63	0.49	0.43	0.45	0.55	0.73	0.66	0.71
	0.11	0.11	0.66	0.61	0.55	0.47	0.52	0.69	0.62	0.94	0.83
48 h	0.11	0.11	0.28	0.50	0.66	0.68	0.80	0.92	1.09	1.19	1.22
	0.11	0.11	0.32	0.48	0.64	0.67	0.86	0.96	1.13	1.16	1.26
	0.11	0.13	0.21	0.38	0.58	0.81	1.04	1.23	1.29	1.45	1.54
72 h	0.07	0.07	0.38	0.61	0.85	0.87	1.34	1.57	1.69	1.88	1.88
	0.07	0.06	0.27	0.52	0.85	0.90	1.32	1.58	1.76	1.93	1.85
	0.10	0.06	0.44	0.63	0.88	0.97	1.53	1.89	1.84	1.98	1.89
<b>PMF</b>											
Concentration ( $\mu\text{g/mL}$ )											
	250	125	62.5	31.3	15.6	7.81	3.9	1.95	0.97	0.48	0
24 h	2.04	0.57	0.73	0.69	0.79	0.82	0.76	0.98	1.04	1.04	0.94
	0.37	0.48	0.60	0.67	0.63	0.89	0.88	0.95	1.06	0.96	0.90
	0.33	0.63	0.77	0.80	0.75	0.66	0.88	0.95	0.86	0.92	0.79
48 h	0.18	0.26	0.37	0.82	1.34	1.74	1.75	1.64	1.88	2.01	1.53
	0.18	0.28	0.39	0.95	1.40	1.72	1.75	1.72	1.79	1.92	1.71
	0.17	0.26	0.37	0.95	1.23	1.56	1.72	1.64	1.72	1.87	1.62
72 h	0.14	0.35	0.40	1.89	2.26	3.15	2.95	2.94	3.04	2.56	2.77
	0.12	0.24	0.40	1.85	2.37	2.71	2.61	2.51	1.98	2.20	2.58
	0.12	0.26	0.33	1.65	2.21	2.71	2.49	2.46	2.27	2.20	2.45

<b>MRG</b>											
<i>Concentration (<math>\mu\text{g/mL}</math>)</i>											
	250	125	62.5	31.3	15.6	7.81	3.9	1.95	0.97	0.48	0
24 h	0.20	0.91	1.12	1.01	0.96	0.93	0.86	0.98	0.93	0.95	0.88
	0.17	0.93	1.09	1.07	0.96	0.88	0.93	0.88	0.95	1.01	1.02
	0.16	0.83	1.10	0.98	0.98	0.99	1.15	1.07	1.12	1.06	0.98
48 h	0.21	1.14	1.62	1.87	1.68	1.70	1.58	1.50	1.58	1.68	1.54
	0.18	1.13	1.67	1.85	1.64	1.58	1.55	1.52	1.59	1.60	1.37
	0.19	1.13	1.52	1.74	1.52	1.60	1.50	1.52	1.67	1.66	1.63
72 h	0.15	1.49	2.67	2.66	2.47	2.23	2.31	2.28	2.30	2.05	2.43
	0.14	1.50	2.65	2.49	2.35	2.51	2.54	2.59	2.52	2.29	2.34
	0.14	1.51	2.27	2.76	2.29	2.49	1.56	0.75	0.95	1.72	2.56

Raw data concerning **Figure 2**: BHK-21 cells infected with CHIKV were exposed to serial dilutions starting from 150 to 1.17 µg/mL for MR and 75 to 0.58 µg/mL for MRG for 72 hours. (4) Viral titer was obtained after five days of cell incubation with the supernatant assay medium. (5) nsP1 viral RNA copies from infected cells incubated with MR or MRG were amplified by RT-qPCR. Myricetin 3-(6-rhamnosilgalactoside) (MRG), myricetin rhamnoside (MR).

**Table 4:** Chikungunya infectious titers expressed as TCID<sub>50</sub>/mL according to Reed and Muench formula obtained after 72 h of the interaction of BHK-21 cell line with CHIKV at MOI of 10<sup>-4</sup> and *M. taxifolia* compounds. Myricetin 3-(6-rhamnosilgalactoside) (MRG), myricetin rhamnoside (MR)

Treatment	Rep *	TCID <sub>50</sub> /mL
Initial titer	1	10 <sup>7.67</sup>
	2	10 <sup>8.33</sup>
MR 75µg/mL	1	10 <sup>5.33</sup>
	2	10 <sup>5.5</sup>
MR 37.5µg/mL	1	10 <sup>6.33</sup>
	2	10 <sup>6.17</sup>
MR 18.7µg/mL	1	10 <sup>6.33</sup>
	2	10 <sup>6.0</sup>
MR 9.3µg/mL	1	10 <sup>6.50</sup>
	2	10 <sup>6.17</sup>
MR 4.68µg/mL	1	10 <sup>7.33</sup>
	2	10 <sup>6.50</sup>
MR 2.43µg/mL	1	10 <sup>6.50</sup>
	2	10 <sup>6.67</sup>
MR 1.17µg/mL	1	10 <sup>6.67</sup>
	2	10 <sup>6.50</sup>
MR 0.58µg/mL	1	10 <sup>7.33</sup>
	2	10 <sup>6.50</sup>
MRG 75µg/mL	1	10 <sup>6.67</sup>
	2	10 <sup>7.33</sup>
MRG 37.5µg/mL	1	10 <sup>6.50</sup>
	2	10 <sup>6.50</sup>
MRG 18.7µg/mL	1	10 <sup>6.50</sup>
	2	10 <sup>6.67</sup>
MRG 9.3µg/mL	1	10 <sup>6.0</sup>
	2	10 <sup>6.0</sup>
MRG 4.68µg/mL	1	10 <sup>6.50</sup>
	2	10 <sup>7.17</sup>
MRG 2.43µg/mL	1	10 <sup>6.33</sup>
	2	10 <sup>6.50</sup>
MRG 1.17µg/mL	1	10 <sup>6.50</sup>
	2	10 <sup>7.0</sup>
MRG 0.58µg/mL	1	10 <sup>7.33</sup>
	2	10 <sup>6.67</sup>

**Table 5:** Ct values were obtained from RNA isolated from VERO and BHK-21 cell lines infected with CHIKV at MOI of  $10^{-4}$  at 0, 10, 22, 34, 46, 70, and 106 hours. Myricetin 3-(6-rhamnosilgalactoside) (MRG), myricetin rhamnoside (MR)

Concentration ( $\mu\text{g/mL}$ )	MR		Concentration ( $\mu\text{g/mL}$ )	MRG	
	$Ct_1$	$Ct_2$		$Ct_1$	$Ct_2$
150	36.298	36.186	75	30.557	30.505
75	26.052	25.870	37.5	24.399	24.833
37.5	25.524	25.586	18.75	23.971	23.706
18.75	24.660	24.723	9.37	23.117	23.071
9.37	24.080	23.946	4.68	22.396	22.850
4.68	23.745	23.780	2.34	22.006	22.063
2.34	21.991	22.055	1.17	21.389	21.454
1.17	21.405	21.683	0.58	20.517	20.378
0	UD*	UD	0	UD	UD

\* UD: undetermined

Raw data concerning **Figure 3** of molecular docking assays of Myricetin 3-(6-rhamnosilgalactoside) (MRG) and myricetin rhamnoside (MR) with nsP3 are available in the ASUS WebStorage cloud storage service at the link: <https://www.asuswebstorage.com/navigate/a/#/s/09DD67A0FF82447985A0CEA05C88675C4>

Raw data concerning **Figure 4** of Molecular dynamics of Myricetin 3-(6-rhamnosilgalactoside) (MRG) and myricetin rhamnoside (MR)-nsP3 complex are available in the ASUS WebStorage cloud storage service at the link: <https://www.asuswebstorage.com/navigate/a/#/s/AAD424377158484993A1D09CF38A2A6F4>