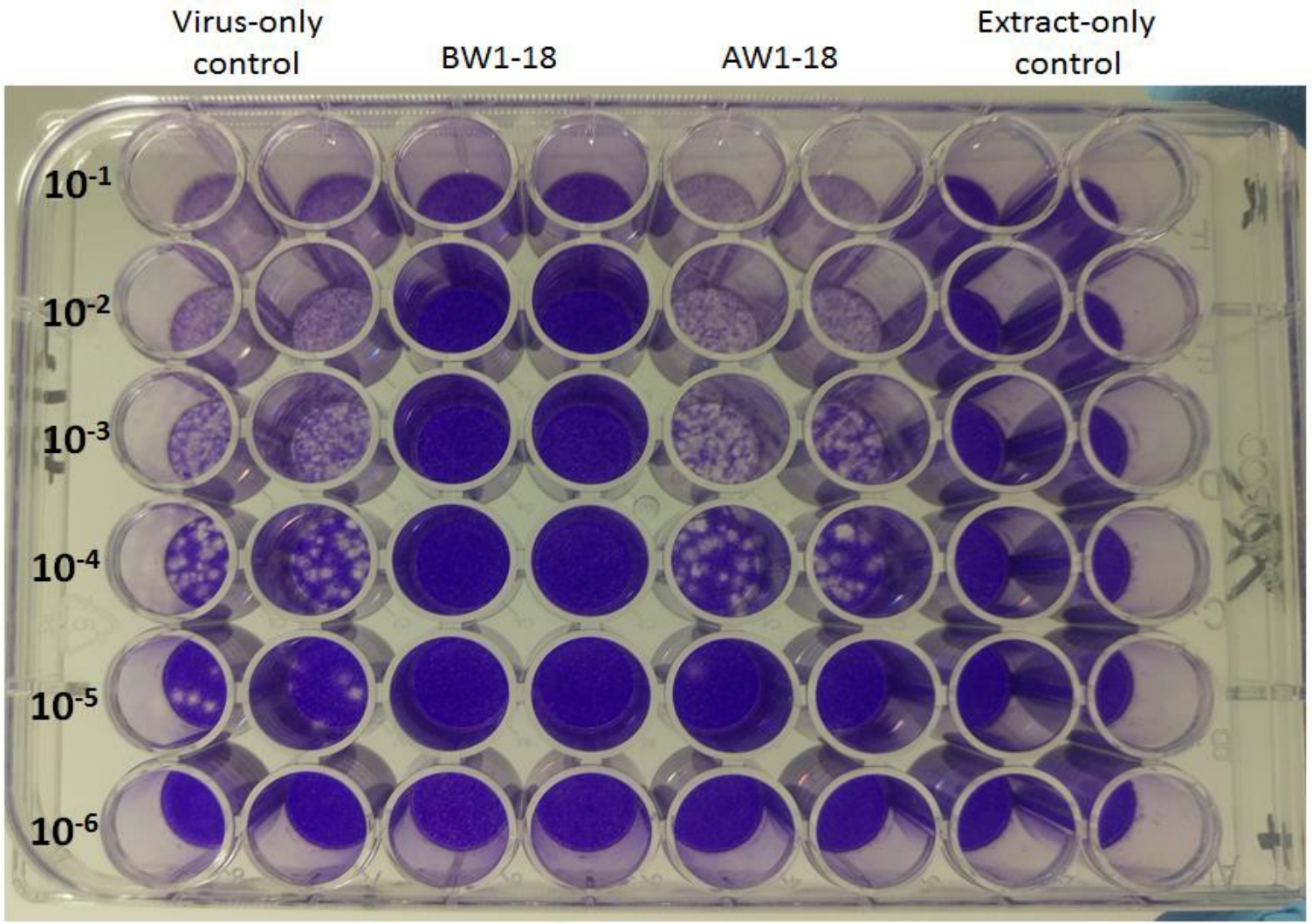

*BW1-18 – Water extract #8473 added 18hours post ZIKV infection
AW1-18 – Water extract #8472 added 18hours post ZIKV infection

Figure 1 – **Plaque assay** plate showing the virus-only control (no extract), virus + extracts A and B (100 mg/mL) added 18hrs post-virus infection of Vero cells and extract only. All samples were performed in duplicate. Serial dilutions of the virus: 10^{-1} to 10^{-6} (in each row as shown above).

In the first set of Zika experiments as indicated in the figure above, both methanolic and water extracts obtained from species 8472 and 8473 were added to Vero cell monolayers infected with ZIKV and left unremoved while samples were taken at different time-points. *i.e*. 2, 4, 6, 15, 18, 24 and 48 hrs. Using plaque assays, the results clearly showed that methanolic and water extracts from species 8473 100% inactivated/killed the virus across all time points. In contrast, the Zika virus was shown to be impervious to species 8472 regardless of solvent/aqueous liquid used or incubation period.

In the second set of experiments, the methanolic extracts obtained from species 8473 were added to Vero cells infected with ZIKV and then subsequently removed at 2 and 24 hrs post infection. The monolayers were rinsed twice with 1x PBS and growth media replaced. Supernatants were harvested at the different time points and stored at -80^{0}C. The assay was stopped at the onset of CPEs and cells were harvested and stored at -80^{0}C.

Plaque assays were carried out on the supernatants and qPCR was performed on the viral RNA extracted from the cells to estimate the concentration of intracellular virus.

It was observed that the samples taken after the extract was left for 24 hours had zero plaques. For the samples that were collected after the extract was left for only 2 hours, the formation of plaques was seen. (Table 1).

Summary of virus titres from Anti-ZIKV assay II

Treatment	Virus titre (Replicate 1) pfu/ml	Virus titre (Replicate 2) pfu/ml	Virus titre (Replicate3) pfu/ml	Description
A0	0	0	0	Sample taken immediately aft ZIKV inoculum removal
A48	1.5×10^6	1.5×10^6	1×10^6	Sample after 48 hours before pu extract
M2	0	0	0	**#8473-MeOH** extract left for 2 before removal – Sample taken immediately
M2 – 2	1.5×10^5	2×10^5	2×10^5	sample taken 2 hrs after extract removed
M2 - 24	2.5×10^6	2.5×10^6	3×10^6	sample taken 24 hrs after extra removed
M2 - 48	3×10^6	3×10^6	5×10^6	sample taken 48 hrs after extra removed
M24	0	0	0	Extract left for 24hrs before ren – Sample taken immediately
M24 - 24	0	0	0	sample taken 24 hrs after extra removed
M24 - 48	0	0	0	sample taken 48 hrs after extra removed
V1	1.5×10^6	4×10^5	1.5×10^6	Virus only control (No extract) sample taken same time as M2
V2	5×10^5	4.5×10^5	4.5×10^5	Virus only control (No extract) sample taken same time as M2
V3	5×10^6	1×10^7	1×10^7	Virus only control (No extract) sample taken same time as M2
V4	2.5×10^7	3×10^7	2.5×10^7	Virus only control (No extract) sample taken same time as M2
Extract unremoved	0	0	0	Extract unremoved throughout t experiment - sample taken same time as M
Extract unremoved	0	0	0	Extract unremoved throughout t experiment - sample taken same time as M
Extract unremoved	0	0	0	Extract unremoved throughout t experiment - sample taken same time as M
W2	0	0	0	**#8473-Water** extract left for 2h before removal – Sample taken immediately
W2- 2	1×10^5	1×10^5	1×10^5	sample taken 2 hrs after extract removed
W2- 24	2.5×10^5	5×10^5	2.5×10^5	sample taken 24 hrs after extra removed
W2- 48	1×10^6	1×10^6	1×10^5	sample taken 48 hrs after extra removed
W24	0	0	0	Extract left for 24hrs before ren – Sample taken immediately
W24- 48	0	0	0	sample taken 48 hrs after extra removed