# Antiviral potency of an extract from *Nerium oleander*

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## INTRODUCTION

Targeting of multiple filovirus species with a single therapeutic is a highly coveted goal. Recent approaches using chimeric antibodies with dual specificities have demonstrated therapeutic efficacy in murine models against EBOV and SUDV, with extended efficacy against BDBV, TAFV and RESTV *in vitro*. The breadth of efficacy, however, is still constrained by genus as efficacy against *Ebolavirus* members was not enjoyed with *Marburgvirus* members. PBI-05204 is a cardiac glycoside-containing extract from *Nerium oleander* that has demonstrated antiviral efficacy and is a cancer therapeutic in clinical trials. PBI-05204 and oleandrin, a cardiac glycoside that is one of the chief therapeutic agents in the PBI-05402 extract, are assessed for antiviral efficacy.

## OBJECTIVES

Assess broad spectrum potency of extract from *Nerium oleander.*

## BACKGROUND

Cardiac glycosides are used therapeutically to correct irregular heart rhythms and congestive heart failure. The cardiac glycoside oleandrin, as well as an oleandrin-containing extract of *Nerium oleander* known as PBI-05204, have also been shown to have antitumor potency[1,2] *in vitro* and *in vivo*. Furthermore, PBI-05204 has been used in phase I and II clinical trials against solid tumors and did not result in any significant cardiotoxicity[3,4]. Interestingly, oleandrin contained within the PBI-05204 *N. oleander* extract has been shown to cross the blood-brain barrier in mouse models[5].

Recent studies have uncovered potential roles for cardiac glycosides as antiviral drugs against HIV, CMV and HSV through a variety of mechanisms[6-9]. Cardiac glycosides disrupt cellular $Na^+$-$K^+$-ATPase, an enzyme that is essential for maintaining the intracellular balance of calcium in smooth muscle cells in the heart by regulating the flow of sodium and potassium ions. Depressed Na+-K+-ATPase activity therefore leads to dysregulated calcium channel function. The indirect inhibition of calcium regulation by cardiac glycosides suggests that these drugs may also be effective against filoviruses as calcium channel blockers have demonstrated efficacy against filoviruses[10-12]. Calcium channel blockers have been demonstrated to mediate their antiviral effect by inhibiting new virus particles from budding[12].

## MATERIALS AND METHODS

A defined extract from *Nerium oleander* and purified oleandrin were used to pretreat Vero cells prior to and post-infection with MARV and EBOV. An immunofluorescence-based assay was used to determine antiviral efficacy 48hr post-infection. For passaging experiments, Vero cells were infected in the presence of PBI-05204 or oleandrin then supernatant was collected 48hr later. The supernatants were then assayed for the presence of infectious virus. An EBOV minigenome was used to assess viral transcription and replication in the presence of PBI-05204 or oleandrin.

## RESULTS

PBI-05204 and oleandrin fully inhibited MARV and EBOV infection in Vero cells. No infectious progeny virus was recovered from supernatants of cells infected with EBOV or MARV when treated with PBI-05204 or oleandrin. Neither virus transcription or replication were inhibited by treatment with PBI-05204 or oleandrin, indicating the inhibition does appear to be linked to viral polymerase functions. Preliminary results also indicate PBI-05204 and oleandrin have antiviral efficacy against other enveloped viruses, demonstrating a broad antiviral profile.

## CONCLUSION

The broad spectrum efficacy we've presented may be especially critical as certain therapeutic elements within the PBI-05204 botanical drug can be found to accumulate in the CNS, which is essential for viruses that have demonstrated neuropathic effects.

## DISCUSSION

- Oleandrin and PBI-05204 exhibit antiviral efficacy that appears consistent with EBOV, MARV inhibitors that prevent budding of new virions.
- PBI-05204 has very similar activity profile to oleandrin, suggesting oleandrin is active compound in PBI-05204 extract.
- Ability of oleandrin to cross blood-brain barrier and efficacy against neutrotropic alphaviruses suggests PBI-05204 has therapeutic potential.

## ACKNOWLEDGEMENT

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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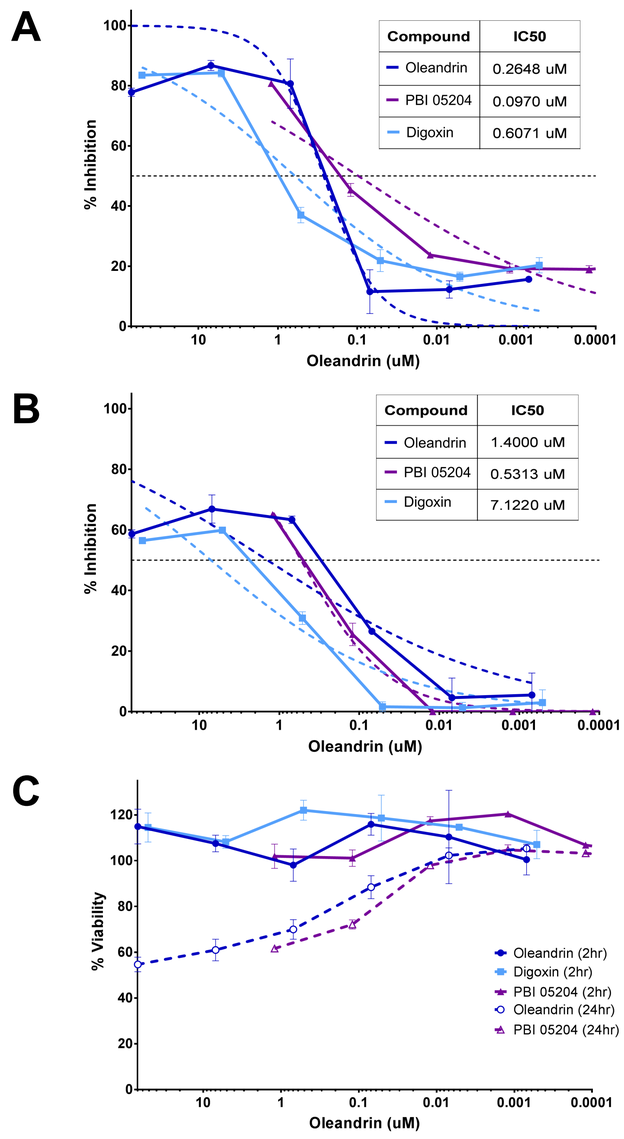


**FIGURE 1. Cardiac glycosides inhibit EBOV and MARV *in vitro*.** Vero E6 cells were treated with oleandrin, digoxin or PBI-05204, an oleandrin-containing plant extract, for 1hr pre-infection then infected with EBOV/Kik (A, MOI=1) or MARV/Ci67 (B, MOI=1) in the presence of compounds. After 1hr, inoculum and compounds were removed and fresh medium added to cells. 48hr later, cells were fixed and immunostained to detect cells infected with EBOV or MARV. Infected cells were enumerated using an Operetta. C) Vero E6 cells were treated with compound for 2hr or 24hr. ATP levels were measured by CellTiter-Glo as an indicator of cell viability.

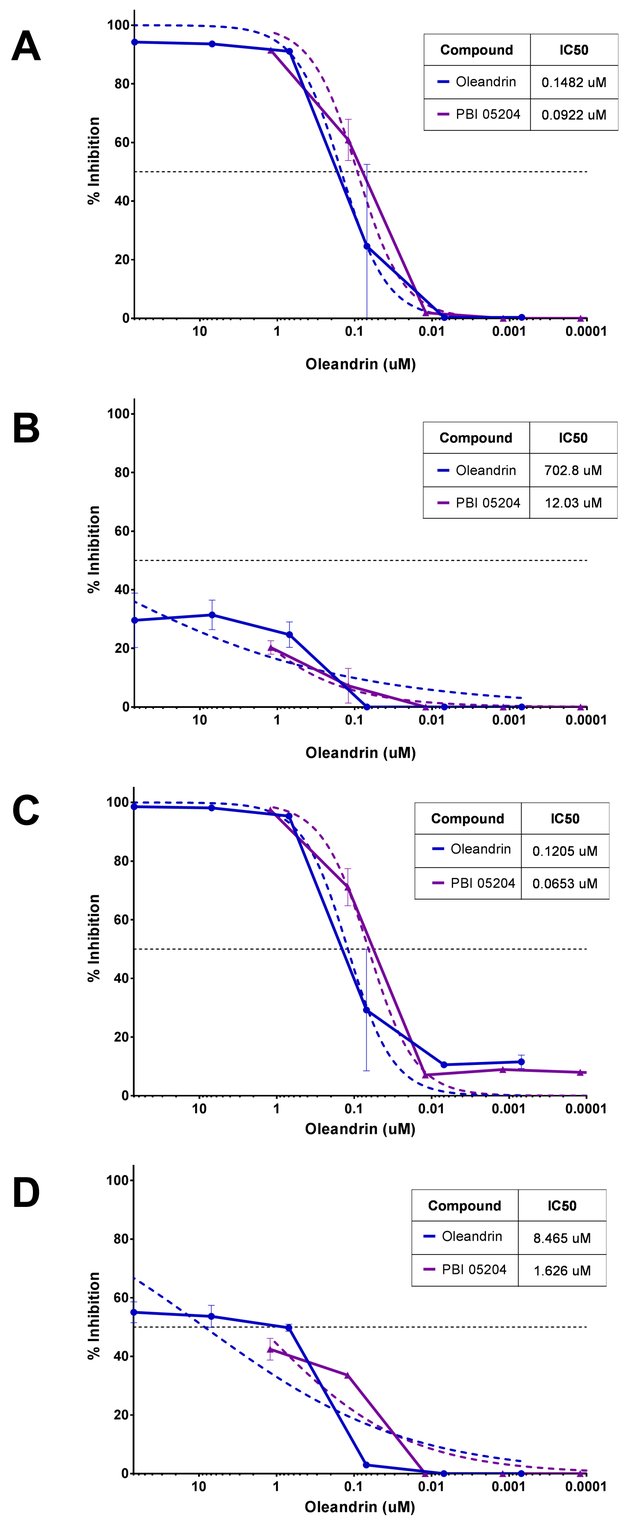


**FIGURE 2. Cardiac glycosides inhibit virus when treated shortly after infection.** Vero E6 cells were infected with EBOV (A,B) or MARV (C,D). After 1hr, virus was removed and oleandrin or PBI-05204 was added immediately to the cells for 2hr (A,C) after which compounds were discarded and cells were returned to medium. Alternatively, compounds were added 24hr post-infection and incubated for 24hr (B,D). At 48hr total, infected cells were analyzed as in Figure 1.

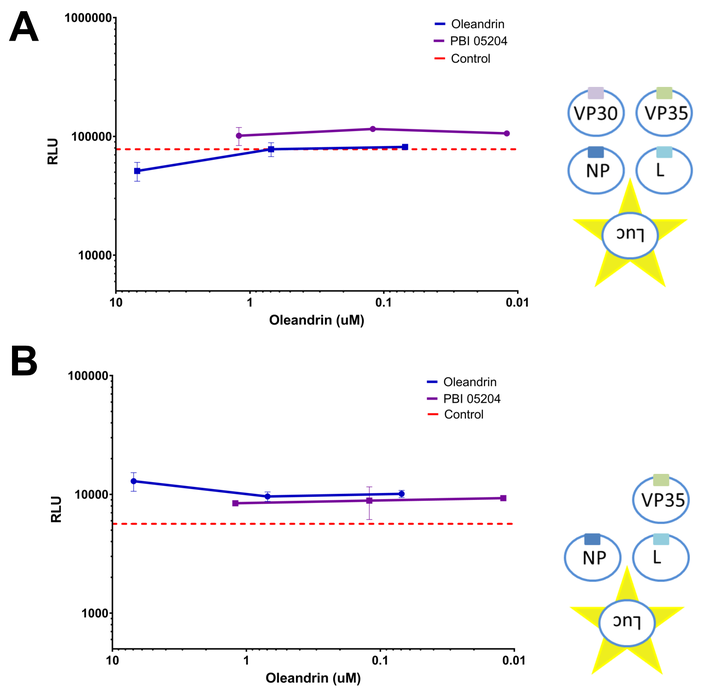


**FIGURE 3. Cardiac glycosides do not inhibit EBOV transcription or replication.** An EBOV minigenome was utilized to determine the efficacy of oleandrin or PBI-05204 in inhibiting virus transcription (A) or replication (B). BSR/T7 cells were transfected with DNA plasmids encoding for T7 polymerase-driven expression of EBOV L, NP, VP35, VP30 (for transcription) and a luciferase reporter in antisense orientation. Plasmids were a kind gift from E. Mühlberger.

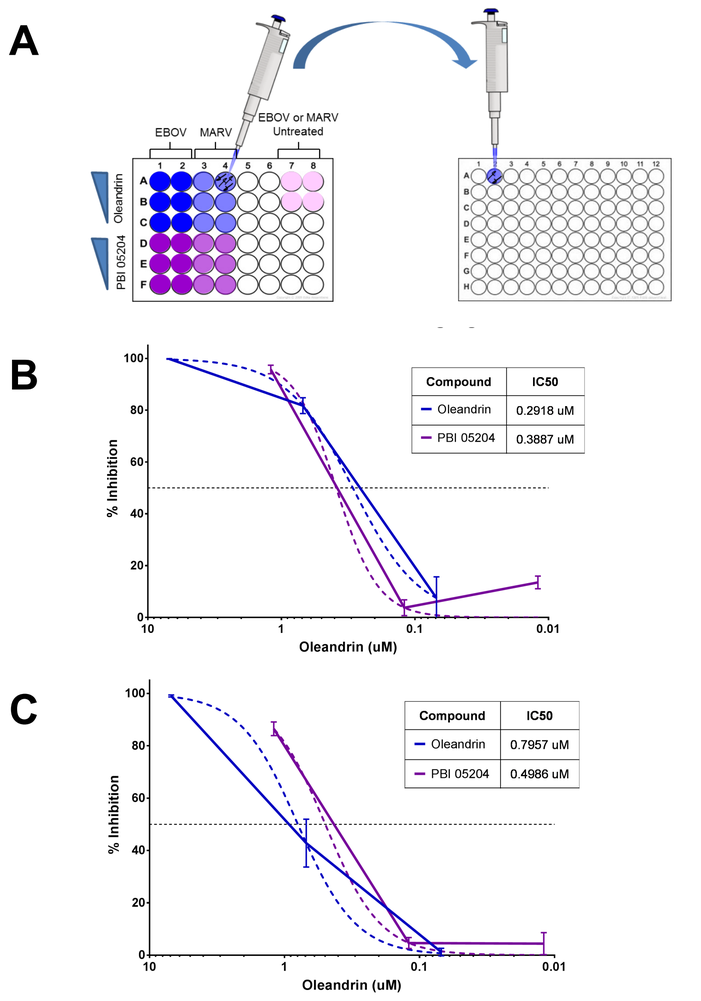


**FIGURE 4. Treatment with cardiac glycosides inhibits the production of infectious progeny.** Vero E6 cells were infected with EBOV or MARV in the presence of oleandrin or PBI-05204. After 1hr, virus was removed, fresh medium was added and cells were incubated for 48hr. Supernatants from infected cell cultures were passaged onto fresh Vero E6 cells, incubated for 1hr, then discarded (as depicted in A). Cells containing passaged supernatant were incubated for 48hr. Cells infected with EBOV (B) or MARV (C) were detected as described previously. Control infection rates were 66% for EBOV and 67% for MARV.

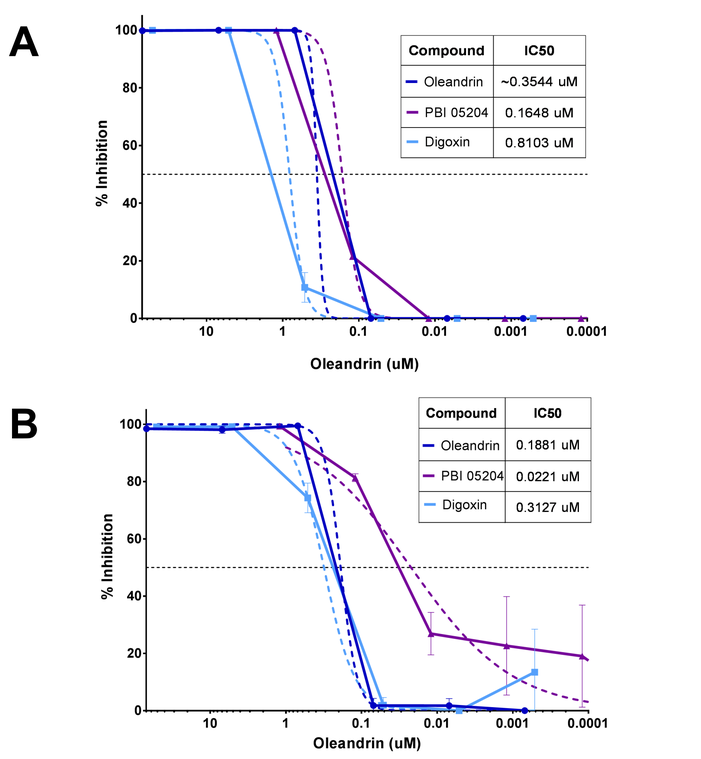


**FIGURE 5. Cardiac glycosides are effective against alphavirus infection.** Vero E6 cells were infected with Venezuelan equine encephalitis virus (A, MOI=0.01) or Western equine encephalitis virus (B, MOI=0.1) in the presence or absence of indicated compounds. After 1hr, inoculum and compounds were removed and fresh medium added to cells. 24hr later, infected cells were detected as before and enumerated on an Operetta.