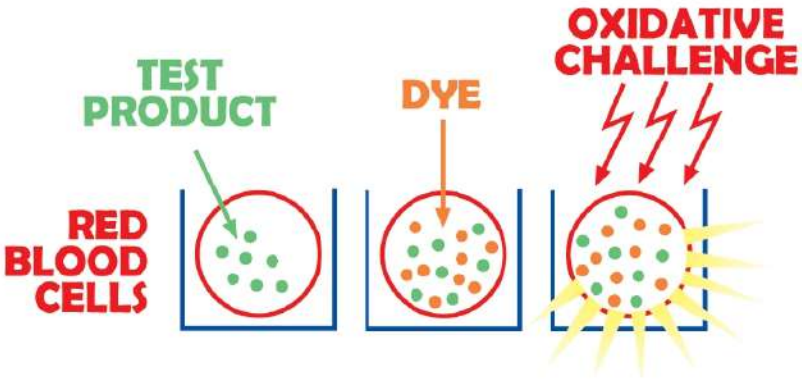
4 Results

4.1 Cellular Antioxidant Protection

The rationale[i] behind the method that we use is important: It allows assessment of anti-oxidant potential in a method that is comparable to the ORAC test, but only allows measurement of antioxidants that are able to cross the lipid bilayer cell membrane, enter the cells, and provide biologically meaningful antioxidant protection under conditions of oxidative stress.

We developed the CAP-e bioassay specifically to work with natural products and ingredients.[ii] The method has been used on multiple types of natural products and ingredients, published in the peer-reviewed scientific literature.[iii iv v vi vii viii]

As a model cell type, we use the red blood cell (RBC). This is an inert cell type, in contrast to other cell types such as PMN cells (often used for subsequent testing of anti-inflammatory effects of natural product and extracts). We developed the red blood cell-based assay particularly to be able to assess antioxidants from complex natural products in a cell-based system, as well as help interpret subsequent data from more complex cellular models.



Human RBC are washed repeatedly in physiological saline, and then exposed to the test products. During the incubation with a test product, any antioxidant compounds able to cross the cell membrane can enter the interior of the RBC. Then the RBC are washed to remove compounds that were not absorbed by the cells, and loaded with the DCF-DA dye, which turns fluorescent upon exposure to reactive oxygen species. Oxidation is triggered by addition of the peroxyl free radical generator AAPH. The fluorescence intensity is evaluated. The low fluorescence intensity of untreated control cells serves as a baseline, and RBC treated with AAPH alone serve as a positive control for maximum oxidative damage.

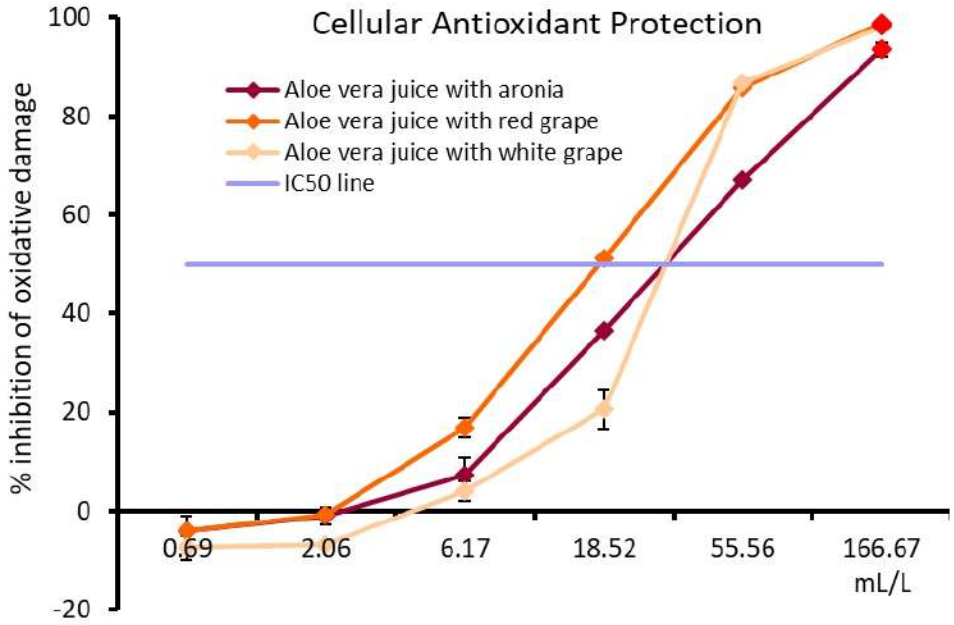


Figure 1. Cellular Antioxidant Protection is compared for the aloe vera juice with aronia, aloe vera juice with red grape, and the aloe vera juice with white grape. The percent inhibition of cellular oxidative damage is shown as the average ± standard deviation of duplicate data points for each dose of test product.

Note: Red data points indicate cell lysing. This was seen for the highest dose of the 3 test products. Cell lysing can happen at higher doses of test products that for various reasons are not well tolerated by the live cells. Lysing can be caused by unfavorable pH, sugar or salt concentration and other factors.

5 Further work

Further in vitro work may include various tests for:

- Inhibition of free radical production by inflammatory cells;
- Mitochondrial function under healthy and stressed culture conditions;
- Effects on nitric oxide production;
- Immune support.

A clinical pilot study may document serum antioxidant capacity after consuming the product compared to placebo. The blood testing may include a clinical adaptation of the CAP-e assay, previously used in published work from NIS Labs on antioxidant-rich natural products.[iii,v]