Effect of Aqueous and Methanol Extracts of *Tradescantia zebrina* and *fluminensis* on Human Cells

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Abstract Summary

Tradescantia zebrina and *Tradescantia fluminensis* have medicinal properties. Extracts of these plants may have anticancer characteristics. Assays were preformed that measured doubling time and clonogenic survival of SCC-13y (squamous cell carcinoma), HFF-1 (human foreskin fibroblasts), and A549 (lung adenocarcinoma) cells. Proliferation inhibition was determined via growth curve analysis. Compared to the negative control of sterile water, cancer cell proliferation was decreased with the addition of *T. zebrina treatments*. This study confirms the general inhibitory effects of *T. zebrina* and *T. fluminensis* extracts on cancerous and non-cancerous cells, allowing further research to be conducted involving different cell lines and methods of extraction.

***Figure 1.* A)** *T. fluminensis* plant **B)** *T. zebrina* plant

Extraction Methods

Table 1. Comparison between methanol and aqueous extraction methods, along with challenges faced during development and the solutions implemented to solve the problems.

	Aqueous Extraction	Methanol Extraction
Benefits	Simple Procedure Clear Extract Sterile	Definite Concentration Preserves Molecules
Challenges	Indefinite Concentrations	Complex Procedure Murky Extract Non-sterile
Solutions	Used Improved Methanol Extraction Method	More Concentrated Extracts Used 0.22-µm Filter Sterilizer Used Centrifuge Evaporator

Extract Treatment Procedure

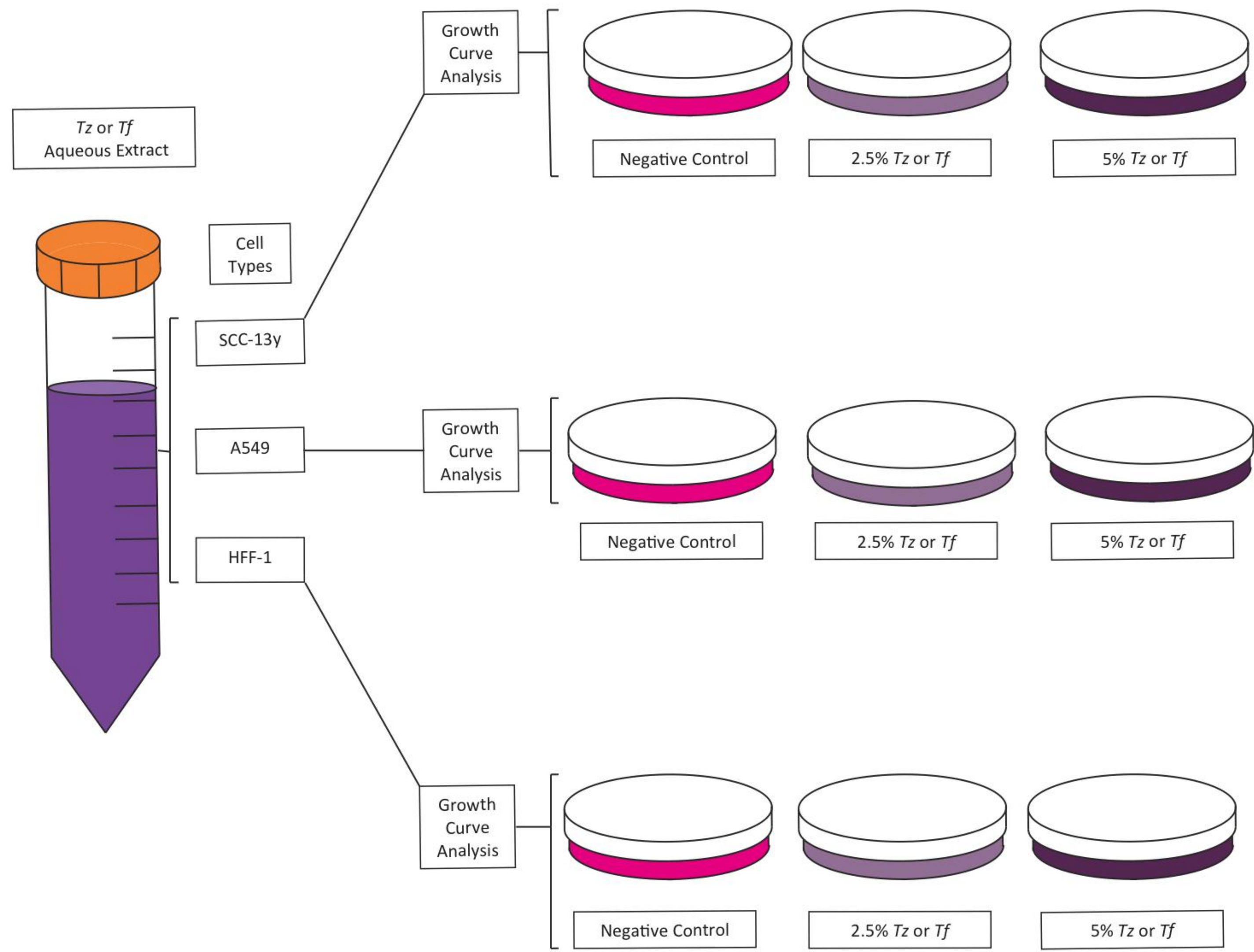
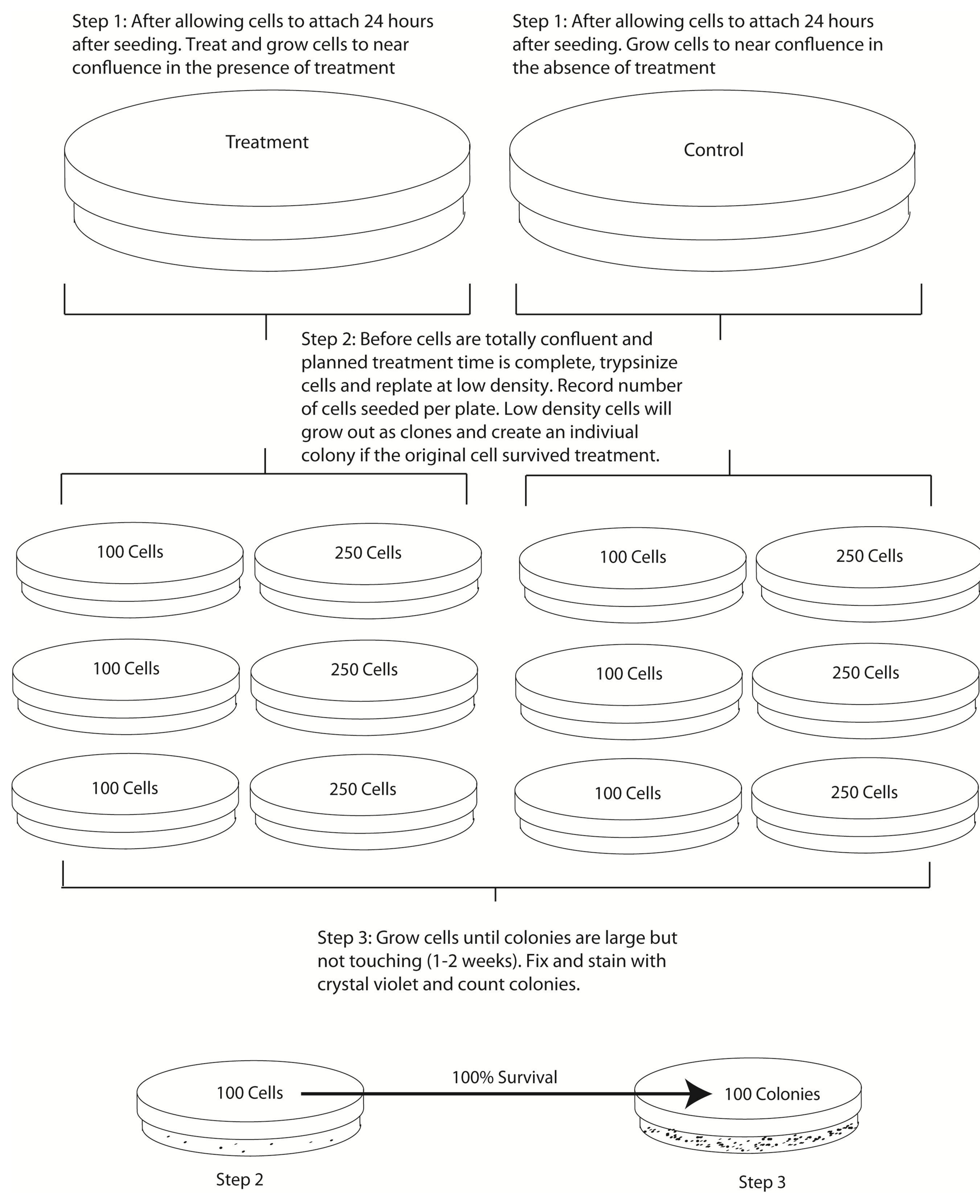


Figure 2 (above). Flowchart of treatment procedure. Aqueous extracts of *Tradescantia zebrina* and *Tradescantia fluminensis* were diluted and applied to SCC-13y cells. Future experiments allow for the use of different concentrations of *T. fluminensis* and *T. zebrina* methanol extract applied to different cell lines. ***Figure 3 (below).*** Clonogenic survival assays were performed using confluent SCC-13y plates of a 2.5% aqueous treatment and a control of sterile water. Each confluent plate was trypsinized and counted using a hemacytometer. Cells were seeded in triplicate for both the experimental and control groups at 100 and 200 cell densities in p60 dishes in DMEM media supplemented in 10% FBS and 1% P/S. Cells were allowed to colonize for one week and were then fixed with 4% paraformaldehyde and treated with crystal violet stain. Stained cells were then counted to determine clonogenic survival rate.

Clonogenic Survival Methods



Preliminary Investigation: Inhibition of Proliferation

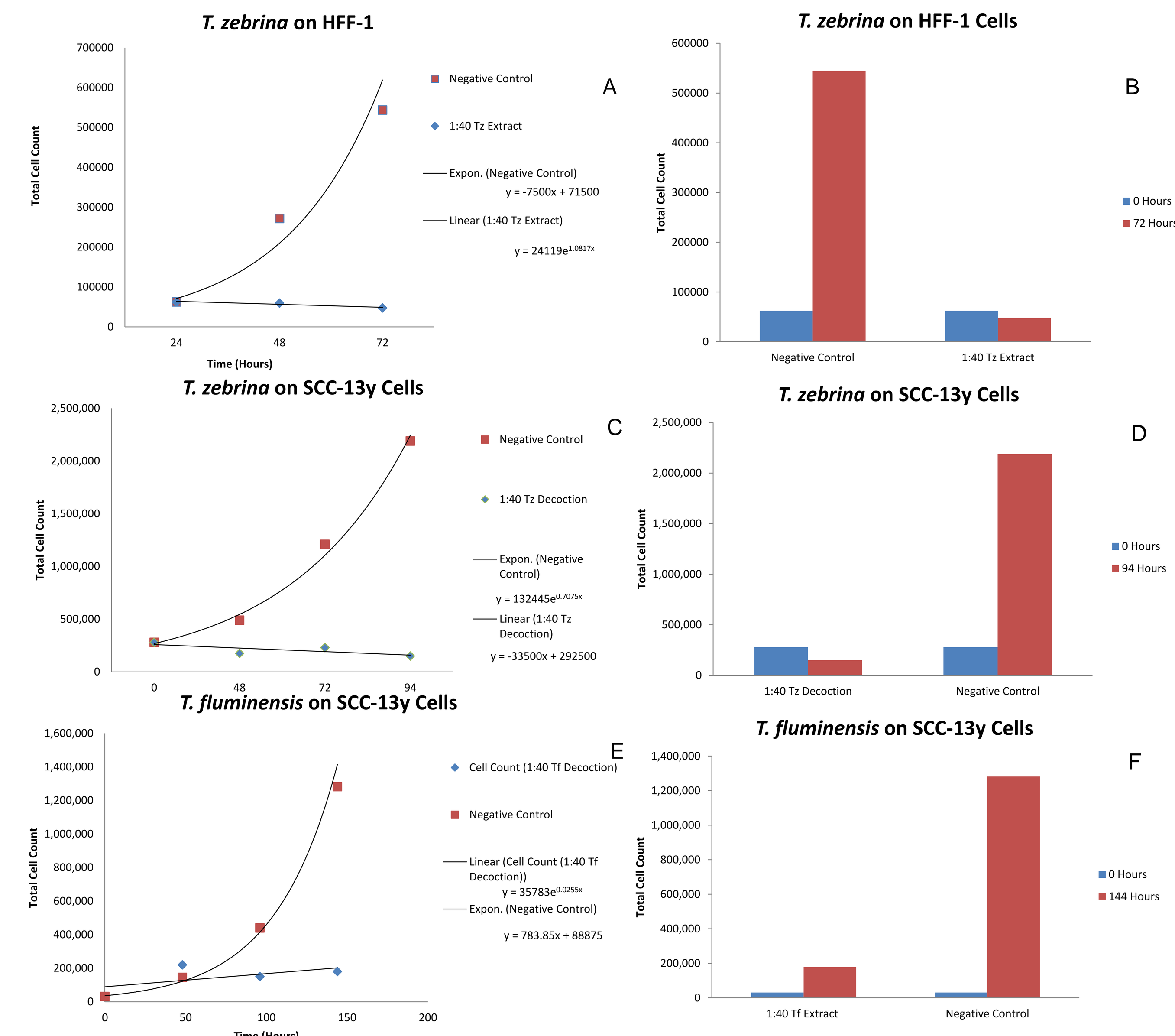


***Figure 4.* A)** Growth curve analysis of a 2.5% *T. zebrina* aqueous extract. ~62,500 HFF1 cells were seeded, treated with *T. zebrina* aqueous extract, and counted 24 and 48 hours post-seeding. **B)** Comparative analysis of 2.5% *T. zebrina* extracts on HFF1 cells as compared to the negative control of sterile water. **C)** Growth curve analysis of 2.5% *T. zebrina* extracts on SCC-13y cells. ~280,000 SCC-13y cells were plated in p60 dishes and treated with 2.5% *T. zebrina* aqueous extracts using equal concentrations of sterile water as a negative control. Cells were harvested and counted at 24, 72, and 96 hours post-seeding **D)** Comparative analysis of 2.5% *T. zebrina* extracts with the negative control of sterile water on SCC-13y cells 0 hours post-seeding and 94 hours post-seeding. **E)** Growth curve analysis of *2.5% T. fluminensis* extracts on SCC-13y cells. ~31,250 SCC-13y cells were plated and treated with 2.5% *T. fluminensis* aqueous extract using sterile water as a negative control. Control and treatment plates were harvested and counted with a hemacytometer after 48, 96, and 144 hours post-seeding in order to generate a growth curve **F)** Comparative analysis of 2.5% *T. fluminensis* extracts with the negative control of sterile water 0 hrs post-seeding and 144 hours post-seeding. Error bars are not shown for all graphs due to a lack of triplicate testing.

Future Goals and Research

1. Clonogenic Survival Assay – to determine the survival rate of cells after the removal of *T. zebrina* and *fluminensis* extract treatment.

2. Zebrafish Toxicity Treatment – to determine if the antiproliferative properties of the extract are due to cytostatic activity within the cell or due to toxicity of the extract.

3. Flow cytometry readings using fluorescence-activated cell sorter of cells treated with *T. zebrina* or *T. fluminensis* extract would generate a cell cycle index that could reveal cytostatic activity within the cell.