# Evaluating medicinal plants for anticancer properties: testing plant extracts for cytotoxicity.

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Graphical Abstract
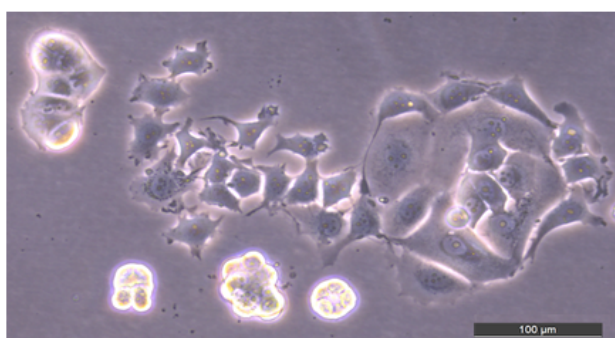
MCF7 breast cancer cells 7 *days in vitro*.

scarlet bush　　　　　　　moringa
Hamelia patens　　　　*Moringa oleifera*

Abstract

Cancer describes a class of diseases which involve the uncontrolled growth of abnormal cells and the spread or metastasis of those cells to other sites in the body. Natural products derived from plants are valuable sources for anticancer drug discovery. The long term goal of this project is to isolate potential anticancer compounds from medicinal plants using bioassay guided fractionation, a process through which components are purified from an extract by multiple rounds of chemical separation and biological activity tests. Towards this purpose, we commenced our research by performing cytotoxicity assays on chemical extracts obtained from plants with medicinal properties or health benefits. The plants included in this study are commonly known as muscadine, scarlet bush, Brazilian pepper tree, anamú, moringa, guanábana, oyster plant, and Okinawa spinach. Plant extracts, prepared with aqueous and/or organic solvents (including dimethyl sulfoxide, ethanol and hexane), were tested on MCF7 breast cancer cells cultured *in vitro*. Methylthiazol tetrazolium (MTT) assays were used to quantify cytotoxicity. Preliminary data indicated the extracts were not cytotoxic at the concentrations tested. On the contrary, extracts from each type of plant improved cell viability. These data provide valuable dosing information regarding extract concentrations for upcoming experiments, including cell invasion assays, which model metastatic processes, and studies on other human cancer cell lines.

Introduction

Treating cancers requires an integrative approach, utilizing multiple therapies that complement one another. Cancer development and progression has been described as exhibiting the following characteristics: (1) maintained proliferative signaling, (2) evasion of growth suppressors, (3) genomic instability and mutation, (4) replicative immortality, (5) avoidance of cell death, (6) tumor-promoting inflammation, (7) activation of invasion and metastatic pathways, (8) induction of angiogenesis, (9) reprogramming of cellular metabolism, and (10) escape from immune destruction [1]. Plants traditionally used for medicinal purposes contain a rich repository of bioactive compounds, which have the potential to therapeutically target these features of cancer biology [2].

Materials and Methods

MCF7 breast cancer cells (American Type Culture Collection) were plated on 96-well plates at a density of 15,000-20,000 cells per well. Cells were cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12, 10% fetal bovine serum, and 1X penicillin/streptomycin.

Plant extracts were prepared from muscadine (*Vitis rotundifolia*), scarlet bush (*Hamelia patens*), Brazilian pepper tree (*Schinus terebinthifolius*), anamú (*Petiveria alliacea*), moringa (*Moringa oleifera*), guanábana (*Annona muricata*), oyster plant (*Tradescantia spathacea*), and Okinawa spinach (*Gynura bicolor*) utilizing aqueous and/or organic solvents [3-10]. Dimethyl sulfoxide (DMSO) was added to extracts at the concentrations listed for the control (Table 1) to improve solubility and cellular internalization. Extracts were then filter-sterilized and diluted in media as described in Table 1. Concentrations varied between extracts because the aim was to maximize extract concentration, not to test uniform extract concentrations.

Cells were first cultured for 48 hours and then treated with plant extracts for 72 hours. Methylthiazol tetrazolium (MTT) assays were conducted to evaluate cytotoxicity. Culture media was replaced with RPMI1640 (without phenol red), 10% fetal bovine serum, and 0.5 mg/mL MTT. Formazan crystals were solubilized in 0.1 N HCl (diluted in isopropanol). Samples were read on a Synergy H1 (BioTek) plate reader set to 570 nm with a 630 nm reference background subtraction.

Table 1: Plant extract concentrations tested

Extract	mg/mL
Control (DMSO)	110 or 154
Anamú Leaves	7.1
Anamú Roots	9.4
Braz. Pepper Bark 75 EtOH/25 Hex	0.1
Braz. Pepper Bark (dried)	1.9
Braz. Pepper Berry (dried)	9.4
Braz. Pepper Berry 100 EtOH	8.8
Braz. Pepper Berry 75 EtOH/25 Hex	2.4
Braz. Pepper Leaves (dried)	9.4
Braz. Pepper Leaves 75 EtOH/25 Hex	3.3
Braz. Pepper Leaves 50 EtOH/50 Hex	20.0
Braz. Pepper Leaves CH2Cl2	0.9
Guanábana Leaves	1.0
Moringa Bark	1.5
Moringa Leaves	1.5
Moringa Seeds	9.3
Muscadine Fruit (dried)	1.4
Muscadine Fruit (whole)	1.4
Muscadine Fruit Outer Shell	39.5
Muscadine Fruit Pulp	28.3
Muscadine Leaves 100 EtOH	13.3
Muscadine Leaves 75 EtOH/25 Hex	10.0
Muscadine Leaves 50 EtOH/50 Hex	7.8
Muscadine Leaves CH2Cl2	3.6
Muscadine Roots 75 EtOH/25 Hex	11.6
Oki. Spinach Leaves Aqueous	9.3
Oyster Plant Leaves 50 EtOH/50 Hex	3.6
Scarlet Bush Leaves	61.7

Each extract was tested using 5-16 replicates in one or two independent experiments. Data from extract-treated cells were compared to that obtained from untreated controls with the same DMSO concentration. Absorbance values were averaged and normalized to controls. Standard deviations of normalized values were calculated. Two-sample, two-tail *t*-tests assuming unequal variances were utilized to calculate *p*-values. In the case of multiple experiments, the largest *p*-value was reported.
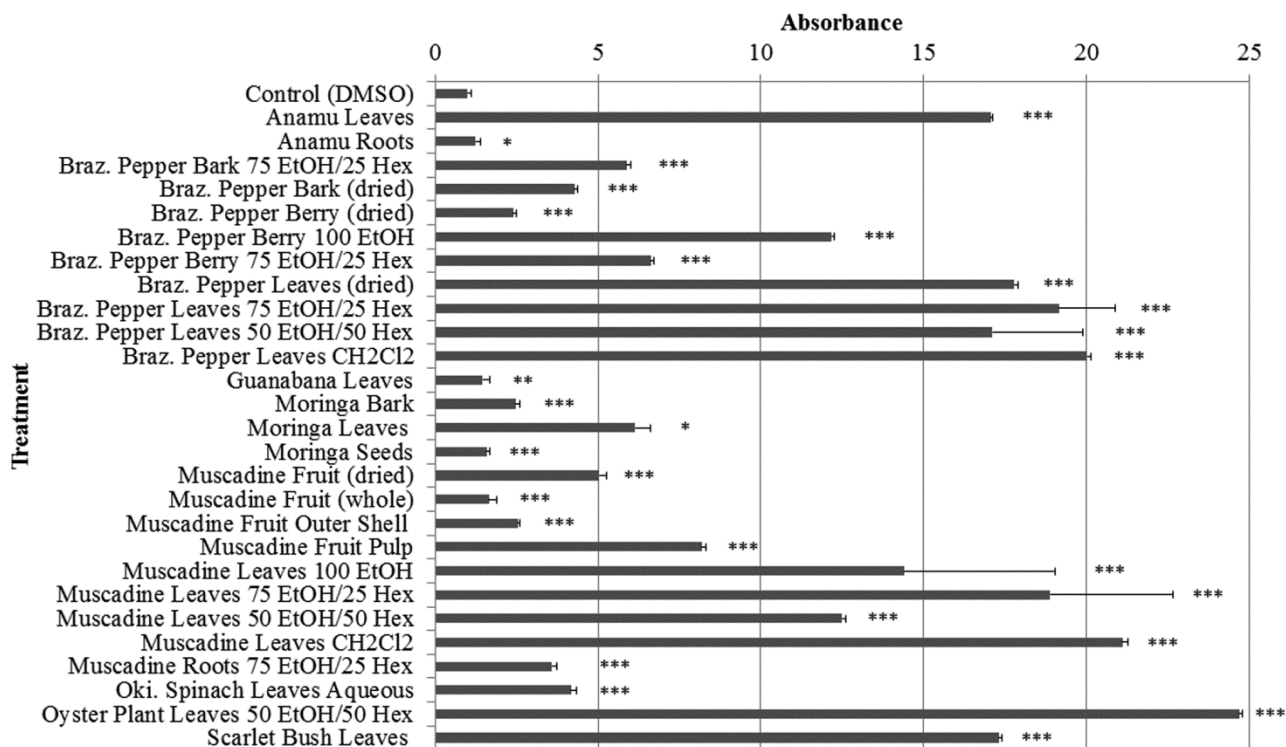
Results and Discussion

Figure 1 summarizes preliminary data regarding the effect of plant extracts on MCF7 breast cancer cells. Extracts were not cytotoxic at the concentrations tested. The greater the absorbance, the higher the concentration of formazan, a purple product generated in live cells by mitochondrial succinate dehydrogenase (SDH) enzyme reduction of MTT. Variability in the data, especially evident in the larger error bars observed at higher values, are primarily attributed to the challenge of consistently solubilizing higher formazan concentrations with manual trituration.

Wells treated with extracts from each type of plant had significantly higher SDH activity during the MTT assay than untreated wells (Fig. 1). This activity is proportional to the number of live cells in a well, which is related to cell survival and cellular proliferation. Hence, overall the extracts reduced cell death. These results are not surprising given that extracts from these plants have been reported to have medicinal effects [reviewed in 3-8]. Moreover, recent studies suggest several of the extracts demonstrate antioxidant activity in chemical reactions [3-8]. In general, antioxidants improve cell viability, including that of cancer cells, which tend to exhibit elevated levels of reactive oxygen species due to metabolic and signal transduction aberrations related to tumorigenesis [11]. Antioxidants provided by the plant extracts could reduce electron leakage during mitochondrial respiration and superoxide formation, increasing the number of live cells in treated samples.

Figure 1: MTT Cytotoxicity Assay. The number of live cells is proportional to absorbance values. Bars indicate the mean absorbance of each treatment normalized to the control. Error bars represent standard deviations. Asterisks correspond to *p*-values: * *p*<0.01, ** *p*<0.001, ***p*<0.0001.

Conclusions

In summary, plant extracts derived from muscadine, scarlet bush, Brazilian pepper tree, anamú, moringa, guanábana, oyster plant, and Okinawa spinach were not cytotoxic to MCF7 breast cancer cells. These findings are relevant, as they indicate the listed concentrations can be used in other assays to study potential anticancer properties present in the plant extracts. Future experiments will test how the plant extracts affect invasion-associated processes, such as cell migration, cell adhesion, and cell aggregation in breast cancer cells, as well as other cancer cell types.

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