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Article

Investigating the anticancer effects of *Uvularia perfoliata*

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SUMMARY

Cancer is one of the leading causes of mortality globally. Current treatments, such as radiotherapy and chemotherapy, have numerous side effects. To create cancer therapies, various studies have investigated the anticancer effects of plants, but present literature fails to mention the potential anticancer effects of *Uvularia perfoliata*. Despite being used by Native American tribes in salves, no scientific investigation has studied the potential medicinal properties of *U. perfoliata*. The objective of this study was to identify whether *U. perfoliata* could be a viable option for future drug discovery research. We hypothesized that if we applied an ethanol extract of *U. perfoliata* to uveal melanoma cells, 70% or more of the cells would die. Although our findings did not match our a priori value for the magnitude of cancer cell killing, the 0.1% concentration *U. perfoliata* did significantly decrease melanoma cell viability ($p < 0.05$). Future testing must be conducted with non-malignant cell lines and higher concentration extracts to understand if *U. perfoliata* possesses anticancer properties. These additional experiments must be conducted because our extracts were significantly more dilute than similar studies. In addition, the cytotoxic properties of *U. perfoliata* may also apply to non-malignant cultures, so future experiments should include non-malignant cell lines as a control. However, this is the first study to highlight the potential anticancer effects of *U. perfoliata*.

INTRODUCTION

In 2020, there were 19,965,054 reported cases of cancer globally; 9,736,520 cases led to death (1). Cancer is a pathological disease characterized by unbridled cellular proliferation and remains one of the leading causes of mortality worldwide. In fact, according to the Global Cancer Observatory, 28.4 million new cases of cancer will appear by 2040 (2).

Unfortunately, conventional cancer interventions, such as chemotherapy, radiotherapy, and surgical procedures, have substantial economic burdens, extensive recovery times, and sporadic inefficacy (**Figure 1**) (3). Due to the numerous side effects of traditional treatments, much research has been done deriving novel anticancer compounds from natural sources. People have studied plants extensively for thousands of years for their medicinal properties. Various cultures have incorporated indigenous flora in traditional medicine for centuries; for example, uses of several medicinal plants are inscribed in the Egyptian Ebers Papyrus, dating back to 1550 BCE (3). In recent years, researchers have been studying medicinal plants for potential anticancer and pro-apoptotic behavior (4). In the 1960s, the potential of a plant-derived cure to cancer was so promising that the U.S. National Cancer Institute systematically evaluated over 35,000 plant species for their potential anticancer properties, leading to the discovery of taxol, which allowed for the development of paclitaxel and docetaxel (5). Numerous current cancer treatments, including paclitaxel and docetaxel, are derived from naturally occurring biological sources, in this case, plants (6). Other natural biologics, such as curcumin, also show great promise as cheaper alternatives to conventional cancer treatments (7-9).

Studies investigating potential new biological therapeutics have approached this by using various solvents to extract compounds such as flavonoids, polyphenols, and alkaloids. For example, a study macerated three plant species in ethanol and water, applying the extract on various human cell lines (5). One of the extracts, vern, inhibited cell proliferation by about 80%.

In this study, we investigated the potential anticancer properties of *Uvularia perfoliata*, a perennial wildflower native to the eastern coast of North America (10). It has known medicinal properties and has been utilized by native groups to treat boils, wounds, and ulcers (10). Nevertheless, existing literature does not mention *U. perfoliata* and its potential anticancer properties (**Figure 2**). This gap in scientific knowledge, combined with its known therapeutic uses in traditional medicine, motivated our investigation. In this study, we sought to determine if *U. perfoliata* could exhibit anticancer activity when extracted in ethanol. We chose ethanol in this study for its enhanced ability to dissolve polar and nonpolar molecules (11-13). We hypothesized that an ethanol extract of *U. perfoliata* would yield 70% cell death on treated uveal melanoma cells (30% cell viability). All four of our *U. perfoliata* extracts failed to meet our 70% cell death *a priori* value. However, the 0.1% concentration extract had a statistically significant effect on cell viability ($p < 0.05$).

RESULTS

To assess if *U. perfoliata* displayed any anticancer activity, we treated uveal melanoma cells with four different *U. perfoliata* extracts. We chose uveal melanoma cells because current research suggests that uveal melanoma cell models are receptive to natural compounds. In fact, pristimerin, oridonin, and zeaxanthin are effective anticancer treatments against uveal melanoma cells (14). We treated the cells with four different concentrations of *U. perfoliata* extract created using 95% denatured ethanol: 0.1%, 0.05%, 0.025%, and

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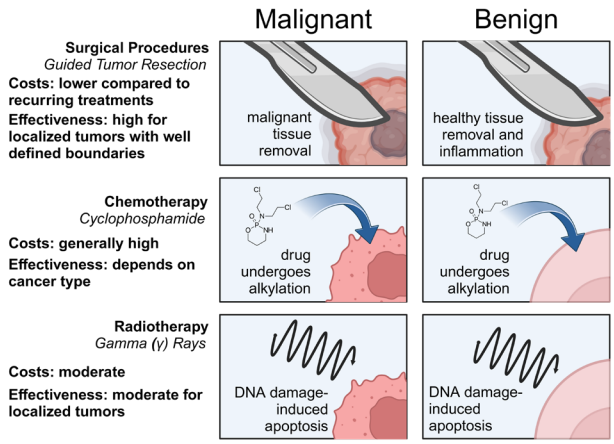

Figure 1: General trends in the efficacy and cost of cancer treatments. Comparison matrix displaying relative cost and effectiveness of widely used cancer treatments. Created in BioRender. Kang, E. (2025) https://BioRender.com/o13c427

0.0125%. These concentrations represent the approximate final percentage of *U. perfoliata* plant material in the cell-media solution. We used several controls to ensure that obtained results were accurate. Puromycin, a cytotoxic antibiotic, was the positive control. We compared the cell viability of an ethanol solution with the same ethanol concentration as the extracts. If the cell viability of the ethanol control significantly differed from the untreated cells, this would mean that the ethanol in our extracts interfered with cellular proliferation. Since the ethanol control did not significantly differ from the untreated cells, we were able to confirm that the concentration of ethanol in our extracts did not exceed the threshold for cytotoxicity, roughly 1% in an *in vitro* environment ($p = 0.3277$). We also included cell-free wells with media and Phosphate-Buffered Saline (PBS) to serves as background controls. We used the 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) cell-viability assay to measure cell viability.

Findings of the MTT assay on uveal melanoma cells

The ethanol control had no effect on cell viability (**Figure 3**). As concentration of *U. perfoliata* increased, cell viability decreased exponentially (**Figure 4**). The 0.01% extract was found to significantly reduce viability of uveal melanoma cells compared to the negative control (Kruskal-Wallis test, $p < 0.05$)

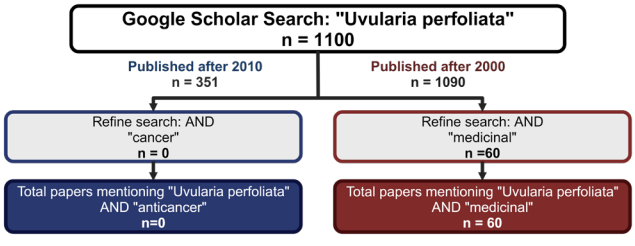

Figure 2: Number of research papers mentioning *Uvularia perfoliata*. Flowchart showing the results from a literature search in Google Scholar, with refining keywords denoted with quotations. Two identical Google scholar searches were conducted using specific keywords appended with "AND". Created in BioRender. Kang, E. (2025) https://BioRender.com/o13c427.

(**Figure 5**). The effects on cell viability of the 0.05%, 0.025%, and 0.0125% extracts were not statistically significant when compared to the negative control ($p > 0.99$). The cell viability of the puromycin-treated cells was significantly lower than the cell viability of untreated cells ($p < 0.05$). The cell viability of cells treated with the puromycin was 78%, while the 0.1% extract had a similar effect ($p > 0.99$). The viability of the cells treated with the 0.05%, 0.025%, and 0.0125% extracts was significantly higher than the puromycin control ($p < 0.05$) (**Figure 5**). In other words, the 0.1% concentration extract had cytotoxic properties similar to the puromycin control.

DISCUSSION

We aimed to determine if *U. perfoliata* could be a suitable candidate for future anticancer drug discovery research. Although our findings did not satisfy our *a priori* value of 70% cell death, our findings are promising because the 0.1% extract displayed a moderate effect on cell viability when compared to the negative control, which was similar to that of puromycin. The effect on cell viability of the 0.05%, 0.025%, and 0.0125% extracts were not statistically significant when compared to the negative control

One significant limitation in our research is that the puromycin control did not effectively kill the melanoma cells. In future experiments, we must ensure the potency of any positive control. We recommend running a mini study to determine the approximate concentration of puromycin needed to achieve full cytotoxic effects. Another limitation is that we did not assess the effects of *U. perfoliate* extracts on non-cancerous cells. Doing so would allow us to determine if there is a difference between the cytotoxic effects of the extracts on cancerous and non-cancerous cells, otherwise known as tumor selectivity. Determining tumor selectivity is important as *U. perfoliata* may be toxic to healthy cells. If future studies prove intriguing, more targeted studies should be employed.

Several solvents used for extraction, including ethanol, acetone, ethyl acetate, and other cytotoxic compounds, may interfere with the assessment of cell viability by causing cell death themselves. To test whether the ethanol influenced cell viability, we incorporated a 1% ethanol control. We observed no significant effect on cell viability in this control group, suggesting that any observed cell death from the extracts was due to the active compounds from *U. perfoliata*.

An additional limitation of this study was our extraction method. We did not make serial dilutions; instead, we combined varying amounts of ethanol with equal-mass samples of *U. perfoliata*. This approach can result in inconsistent extractions since lower solvent volumes may become saturated and not fully extract all bioactive compounds. Future experiments should opt for serial dilutions to ensure each extract has the intended percentage of potential bioactive compounds. Additionally, we could not fully derive a dose-response curve for uveal melanoma cells due to the absence of a rotary evaporator, leading to more dilution than in similar studies (6). In future phases, a rotary evaporator (or a comparable method) will be used to remove and concentrate the solvent, allowing for a more accurate determination of plant extract concentrations needed to achieve complete cytotoxicity (15).

Future studies should also include a larger number of cancer cell lines. Due to limitations relating to funding and timing, only one cell line could be employed. Testing

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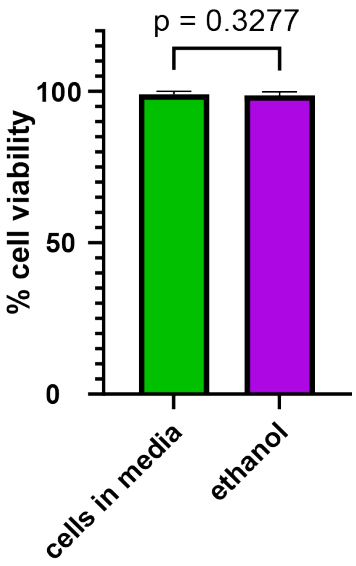

Figure 3: Cell viability of uveal melanoma cells treated with 1% ethanol or media only. We treated cells with the same concentration of ethanol as the plant extracts (<1%). We performed a Mann Whitney test to compare the medians of the two experimental groups. The ethanol treatment did not affect cell viability as there was not a statistically significant difference between the ethanol control and negative control (p = 0.3277).

on several types of cells would provide clues on certain properties associated with *U. perfoliata's* anticancer activity, such as selective toxicity. In addition, future studies should also isolate and identify the individual components within *U. perfoliata*. We recommend that future studies employ spectrometry techniques to characterize the potential anticancer compounds within *U. perfoliata*.

In summary, the 0.1% extract concentration ethanol extract of *U. perfoliata* reduced cell viability in human cancer cells, with a percent cytotoxicity of 10.78% and a cell viability of 89.22%. As no prior anticancer research has been done on this plant, further investigation is needed to fully assess the range and magnitude of this plant's abilities.

MATERIALS AND METHODS

Cell culture

Uveal melanoma cells (AcceGen Biotech Cat.# ABC-TC477S) were maintained at 37°C and 5% CO_2. RPMI 1640 media was used to culture cells, which were passaged twice a week. A biosafety cabinet was used throughout the procedure to avoid contamination

Preparation of plant matter

200 g of *U. perfoliata* (sourced from Dancing Oaks Nursery, Monmouth, Oregon) were washed in a bath of distilled water at 21°C , gently removing any dirt and impurities. The roots were separated from the plant matter and left to air dry for 8 hours.

Preparation of plant extracts

Prepared plant matter was ground into a fine powder with a sterilized mortar and pestle. One gram of the ground powder was added to 10 mL, 20 mL, 40 mL, and 80 mL of 95% denatured ethanol in glass beakers, then stored in 100 mL non-airtight glass containers. Extracts were then placed in a chest freezer at 0°C for 7 days. The solutions were filtered using 0.22 µm Polyethersulfone (PES) syringe filters. Finally, the solutions were diluted with complete media consisting of RPMI-1640 Medium with 10% fetal bovine serum (FBS), 1% L-glutamine, 1% Penicillin-Streptomycin (10,000 U/mL), and 1% MEM Non-Essential Amino Acids Solution in a 1:9 ratio, ensuring the final ethanol concentration did not surpass the 1% cytotoxicity threshold in the final cell culture wells.

MTT assay on uveal melanoma cells

After seeding the cells, we included additional wells containing only phosphate-buffered saline (PBS) or complete media without cells to serve as background controls for the MTT assay. The PBS controls minimized potential evaporation effects, while the media-only controls accounted for baseline absorbance from the medium. 4000 uveal melanoma cells were seeded into each well in 100 µL complete media. After 24 hours of incubation, 10 µL of the extracts were added for final concentrations of 0.0125%, 0.025%, 0.05%, or 0.1% (n = 24). In ethanol control wells, 10 µL of a 10% ethanol solution was added (n = 8). For the puromycin control, 10 µL of puromycin (ThermoFisher Cat.# A1113803) was added (n = 8) for a final concentration of 1 µg/mL. Finally, for the negative control, an additional 10 µL of the media solution was added (n = 8). The plates were then incubated for 24 hours at 37°C with 5% CO_2. After incubation, 10 µL of MTT Reagent (Sigma-Aldrich Cat.# 475989) was added to each well. The plates were incubated again for another 4 hours at 37°C, which was followed by adding 100 µL of solubility solution. Finally, the plates were incubated again under the same conditions for another 24 hours before reading absorbance using the Varioskan LUX Multimode Microplate Reader (sample: 650nm, reference: 570nm). The following formula was used to convert the

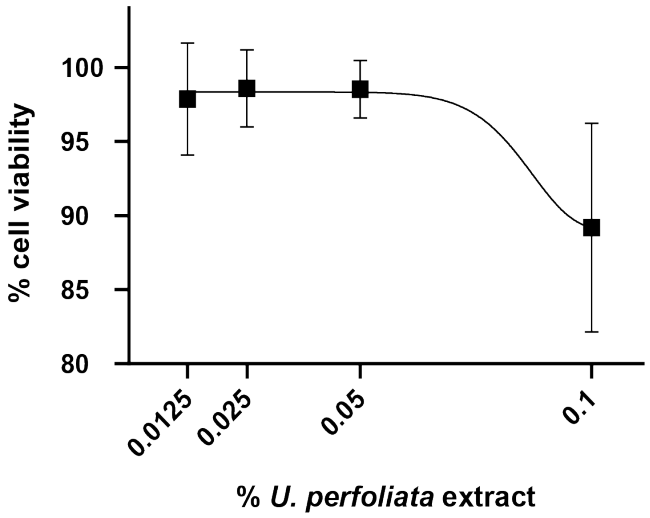

Figure 4: Dose response of *U. perfoliata* extracts on uveal melanoma cells. Dose-response graph showing the exponential decrease in uveal melanoma cell viability with increasing concentration of U. perfoliate extracts (n = 24). Linear regression line and standard error bars are shown. We treated uveal melanoma cells with various U. perfoliata ethanol extracts. X-axis represents the initial ratio of plant(g)/ethanol(mL) of the extracts before we applied them to the cells.

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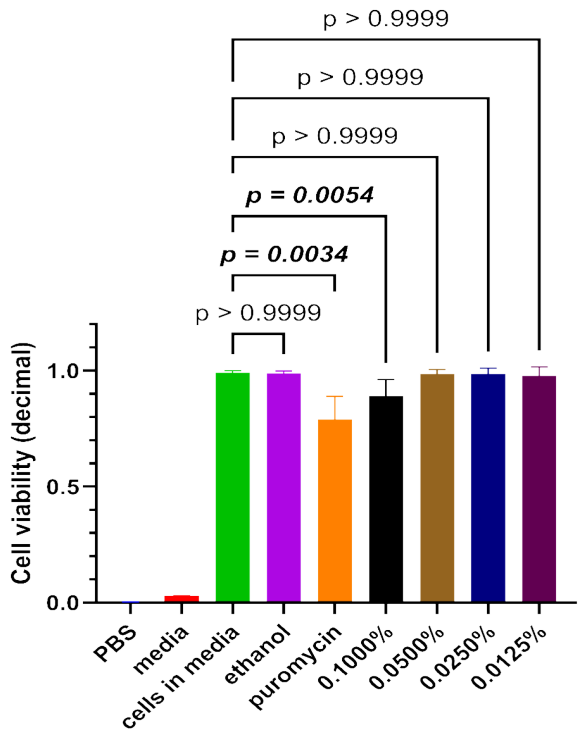

Figure 5: Cell viability as measured by absorbance of the experimental variables. Bar graph displaying mean cell viability of the experimental groups with respective standard deviation error bars (n=24 for extracts, n=4 for controls, and n=8 for the cells in media). Plot shows a pairwise comparison of the negative control and experimental groups (Kruskal-Wallis with Dunn's test). P-values that are notable are bolded. Two identical 96-count well plates seeded with uveal melanoma cells were treated with the experimental variables and controls. On the x-axis, "media" refers to the wells with only complete media added, and "cells in media" refers to the cells in the negative control group, which were untreated. There are no cells in the "PBS" and "media" groups, therefore no significance testing has been conducted for either group.

absorbance values into percent cell viability,

$$\left(\frac{A_{treated\ cells}}{A_{control\ cells}}\right) \times 100 \quad \text{(Equation 1)}$$

where $A_{treated\ cells}$ is the mean absorbance of an experimental group, and $A_{control\ cells}$ is the mean absorbance of the negative control, the cells that received only media.

Statistical testing

A Kruskal-Wallis test was employed to compare the extract groups to the negative control. In addition, Dunn's test was used as the post-hoc test. An alpha of 0.05 was used to determine significance.

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