5-2020

Phytochemical content and anti-breast cancer activity of Kentucky native plants.

Nicole A. McGrath
University of Louisville

Phytochemical content and anti-breast cancer activity of Kentucky native plants.

By:

Nicole A. McGrath

Submitted in partial fulfillment of the requirement for

Graduation *magna cum laude*

And

For Graduation with Honors from the Biology Department

University of Louisville

May 2020

Table of Contents

List of Figures

List of Tables

Abstract

Plants are a proven and rich source of chemical diversity for the development of pharmaceuticals. Plants that are native to Kentucky are an underexplored resource that could lead to advances in treating human diseases such as cancer. Kentucky native plants, especially those with highly diverse or enriched chemical compositions are possible leads for therapeutic drug development. To explore the potential of native Kentucky plants for treatment of breast cancer, a list of prospective target plants was developed based on literature reports. This list was narrowed to 10 tissues for further in-depth chemical analysis including estimation of alkaloids, terpenes, and phenolics, which are categories of plant-derived chemicals that have been reported to have bioactivity. These analyses revealed bloodroot root (*Sanguinaria canadensis*) to have the highest content of alkaloids (65 mg/gDW), purple redbud leaves (*Cercis canadensis*) to have the highest content of terpenes (756 ± 28 mg/gDW), and lemon balm leaves (*Melissa officinalis*) to have the highest content of phenolics (43 ± 3 mg/gDW). Thus, extracts of these three plants were used to screen for activity against the triple negative breast cancer cell line MDA-MB-231 and the endocrine responsive cell line T-47D. The root extract of the bloodroot (*Sanguinaria canadensis*) was found to have the overall highest percent inhibition of cell proliferation in triple negative MDA-MB-231 and endocrine responsive T-47D cancer proliferation (Inhibitory Concentration)$_{50}$ (IC_{50})= 0.0002 and IC_{50} = 0.0005 mg/mL media respectively) when compared to vehicle control. Lemon balm leaves yielded an IC_{50} of 0.0416 for T-47D and IC_{50} = 0.1118 for MDA-MB-231, and purple redbud leaves yielded an IC_{50} of 0.0082 for T-47D and IC_{50} = 0.0869 for MDA-MB-231. Based on these results, Kentucky native plants merit further consideration for research and development of therapeutic drugs. In particular and based on results reported here, bloodroot roots merits more detailed studies for its activity against breast cancer.

Background

Phytochemicals are chemical compounds that are naturally produced by plants. Phytochemical content including phenolics, terpenes, and alkaloids varies among plant species and even within tissue types. These diverse compounds often serve plants in defense against herbivory, competition, or fungal or bacterial infection, and thus display a wide variety of bioactive properties. Based on the inherent bioactive properties of these phytochemicals, many have been used directly or indirectly (*i.e.* drug leads) to treat various human diseases and disorders ranging from Alzheimer's to skin rashes to cancer. A single plant can have an extensive assortment of medicinal properties. For example, the neem tree, *Azadirachta indica*, has a phytochemical profile that provides antidiabetic, antiulcer, insecticidal, antioxidant, and anti-inflammatory properties (1). In some cases, investigating plants to discover new therapeutics has led to single-compound drugs. For example, Arteether and Calanolide A were discovered through phytochemical characterization of traditional medicinal plants (2). Arteether comes from a sesquiterpene lactone isolated from sweet wormwood, *Artemisia anuua*, and is used to treat malaria. Calanolide A, from *Calophyllum lanigerum var. austrocoriaceum*, works against type-1 HIV due to its ability to act as a non-nucleoside reverse transcriptase inhibitor (2). These are just a few examples showing the potential of plants in discovering therapeutic compounds. Clearly, exploring plants to discover new drugs has great potential.

Native plants are those that have occurred in a region for a long period of time and occur naturally, without human intervention; the definition used in this study includes plants introduced from Europe and from similar habitats, for example the dandelion. Kentucky native plants and tissues selected for this project include purple redbud leaves (*Cercis canadensis*), wild ginger rhizomes (*Asarum canadense*), dandelion roots (*Taraxacum officinale*), lemon balm

leaves (*Melissa officinalis*), yarrow flowers (*Achillea millefolium*), pawpaw fruits (*Asimina triloba*), St. John's wort flowering tops (*Hypericum prolificum*), Eastern cedar cones (*Juniperus virginiana*), bloodroot roots (*Sanguinaria canadensis*), and American elderberry fruits (*Sambucus canadensis*).

Reported phytochemical analyses indicate dandelion roots have a phytochemical composition that includes hydroxycinnamic acids, sesquiterpene lactones (3), triterpenes, coumarins, phenolic acids, and flavonoids (4). Yarrow (*Achillea millefolium*) contains borneol, cineole, camphor, and beta-pinene (5), and juniper contains other terpenoids (6). There are benzophenanthridine and protopin alkaloids in bloodroot (7), and anthocyanins and phenolics are contained in American elderberry (8). Thus, the selected plants contain an interesting and diverse array of compounds.

Some of these KY native plants also have recorded medicinal properties. The elderberry, for example, has a high quantity of antioxidants and has been investigated for action against brain cancer cell proliferation through changing the expression of cell cycle checkpoint proteins and inducing apoptosis (9). Some plants are able to inhibit multiple carcinoma types; for example, lemon balm is able to prevent colon cancer proliferation via the production of reactive oxygen species (10) and is also able to diminish lung cancer by inducing apoptosis (11).

The phytochemical content or the bioactivity has been reported for a limited number of Kentucky native plants, but there are no known reports characterizing and comparing the phytochemical content of selected KY native plants coupled with targeted bioactivity assessment against a specific disease such as breast cancer.

Breast cancer continues to be one of the most common causes of female death as it is the most prevalent cancer type (14). Breast cancers are categorized and treated based on whether

they test positive or negative for ER (estrogen receptor), PR (progesterone receptor), and HER2 (human epidermal growth factor receptor 2) gene overexpression. When overexpressed, HER2 increases the spread of malignant cells (15). Because of the metastatic nature of cancer cells, chemotherapeutic drugs are almost always prescribed to breast cancer patients, regardless of the receptor categorization and extent of surgical removal.

There are multiple ways in which chemotherapeutic drugs can mitigate development or proliferation of breast cancer. For example, tamoxifen is a hormone therapy drug that treats ER (estrogen receptor) positive cancers by acting as a competitive inhibitor at the estrogen binding site on estrogenic receptors. Thus, by interfering with ER activation by estrogen, tamoxifen inhibits expression of genes that may further tumor cell growth through estrogen regulation. This results in reduced cell proliferation via G1 cell cycle arrest (13). The phytochemical taxol, inhibits breast cancer proliferation by a different mechanism. Taxol works by inhibiting disassembly of tubulin, thus disrupting normal mitosis (12). Although tamoxifen is a common chemotherapeutic drug, prolonged exposure can lead to development of resistance in breast cancer cells. Thus, exploration of bioactive plants to find alternatives to drugs such as taxol is warranted.

There is evidence that some native KY plant extracts have bioactivity against breast cancer cells. Wild yam root extract decreases cell viability in estrogen receptor positive and negative breast cancers in a concentration-dependent fashion (16). Similarly, in DMBA (7,12 dimethylbenz[a]anthracene)-administered rats, dandelion root extract decreases breast cancer proliferation (17). Thus, the native plants of Kentucky may have great potential for pharmaceutical discovery and development as there are many plants that have been used traditionally for medicinal purposes such as black cohosh (*Cimicifuga racemosa*) and American

ginseng (*Panax quinquefolius*) (18) that can be evaluated further. Extracts of black cohosh have anti-proliferative effects on prostate cancer cells (19) and American ginseng has similar activity against breast cancer cells (20). Many chemotherapeutic drugs used for breast cancer treatment are derived from plant extracts, such as taxol which comes from the Pacific yew. Anacardic acid is derived from plant extracts and is able to inhibit breast cancer cell proliferation in both ER and PR positive and triple negative cell lines (21). Similarly, resveratrol is derived from several plant species, and it suppresses proliferation of breast cancer cells (22).

Clearly, plant-based research has shown great potential in the discovery of therapeutics for human diseases, especially breast cancer. This project reports on the compositions of selected plant tissues native to Kentucky and, based on these results, the effects on breast cancer cell proliferation of three of the selected plant tissues which contained the highest amounts of alkaloids, terpenes, or phenolics.

Materials and Methods

Tissue Collection

A list was made of Kentucky native plants, the plant tissues used medicinally, peak harvest time, historical medicinal uses, and researched phytochemical composition and bioactivity (Appendix Table 1). From, this list of approximately 60, 10 were selected based on availability and potential phytochemical variety and activity.

Targeted plant organs from native plants with potential bioactivity reported in the literature (Table 1) were collected from various sites within the Louisville area during the summer of 2019, and their locations were documented with geographic coordinates. Each sample was weighed, placed in separate labeled bags, and frozen at –80° C. To reduce degradation of phytochemicals and to standardize content to dry weight, each tissue sample was freeze-dried, ground into a powder, and placed into labeled microcentrifuge tubes.

Table 1: Selected KY native plants and their known phytochemical compositions and bioactivity against cancer

Plant Name / Tissue	Scientific Name	Known Phytochemicals	Potential Bioactivity Against Cancer
yarrow	*Achillea millefolium*	achilleine, salicylic acid, azulenes, flavonoids (5)	Yes - pancreatic (28)
wild ginger rhizomes	*Asarum canadense*	aristolochic acid, asarone (27), flavanol and chalcone glycosides (26)	
pawpaw fruit	*Asimina triloba*	phenolic acids, flavonoids (24), isoquinoline alkaloids (25)	
purple redbud leaves	*Cercis canadensis*	anthocyanins	
St. John's wort flowering tops	*Hypercium perforatum*	tannins, flavonoids, hypericin, hyperforin, triterpenes (23)	
juniper cones	*Juniperus virginiana*	flavanoids, tannins, juniperin (6)	Yes - colon (30)
lemon balm leaves	*Melissa officinalis*	citral, citronellal, jasmonic and salicylic acid (29)	Yes - colon (10), lung (11)
American elderberry fruits	*Sambucus canadensis*	anthocyanins, rutin (8)	Yes - brain (9)
bloodroot roots	*Sanguinaria canadensis*	alkaloids, sanguinarine, benzophenanthridine (7)	
dandelion roots	*Taraxacum officinale*	hydroxycinnamic acids, sesquiterpene lactones (3), triterpenes, coumarins, phenolic acids, flavonoids (4)	Yes - breast (17)

Chemicals and Reagents

All chemicals and reagents used were the highest research grade possible and were purchased primarily through VWR (Radner, PA) or Sigma-Aldrich (St. Louis, MO) unless otherwise noted.

Protein Assay

Protein content was estimated using a Bradford assay (62) in 96-well plate format. A protein extract was made by adding 1 mL 0.1 M Tris-HCl (pH 8.0) to 0.1 g dried sample powder in 1.5 ml microcentrifuge tubes. A micro-pestle was used to grind the material, then samples

were centrifuged at 3000 × *g* for 4 min at 4° C. The supernatant was transferred to a clean microcentrifuge tube and dilutions with factors of 2× and 10× were made in additional microcentrifuge tubes. All standards, samples, and dilutions were performed in triplicates. A standard curve was generated using bovine gamma-globulin (γ-globulin) at concentrations ranging from 250 µg/mL to 2 mg/mL with a line of best fit relating protein concentrations and absorbance. The Bradford assay utilized 0.010 mL of sample or standard and 0.300 mL of Coomassie Plus Protein Reagent (Thermo Scientific, Rockford, IL) and absorbance was read at λ = 595 nm according to the manufacturer's instructions.

Starch Assay

To estimate the starch content of selected plant tissues, extracts were made as described for protein assays except the extraction solvent was ethanol/water/acetic acid (75/25/1; v/v/v). The starch assay was performed as described in (61) and scaled to a 96-well plate format. The concentrations of starch within the plant tissues were estimated compared to a linear fit model from potato starch standards ranging from 200 mg/mL to 1000 mg/mL. Each 0.1 mL sample or standard was added to the plate in triplicate with 0.150 mL of iodine reagent and mixed well. Absorbance values were read at λ = 620 nm.

Phenolics Assay

Sample extracts for total phenolics estimations were as described for the protein assay except 1% HCl in methanol (v/v) was used as the extraction solvent. Phenolics concentrations of the targeted plant tissues were based on a linear fit model with gallic acid standards ranging from 0.100 mg/mL to 0.500 mg/mL. The phenolics assay described by Slinkard and Singleton (63) was adapted to 96-well plate format where 0.008 mL of sample or standard was added to wells in

triplicate before adding 0.041 mL 1:10 diluted Folin-Ciocalteau reagent, 0.033 mL 7.5% sodium carbonate, and 0.120 mL deionized water. Samples and standards were mixed and absorbance was read at $\lambda = 765$ nm.

Anthocyanins Assay

Determination of anthocyanin concentration was based on Cheng and Breen (64) and Giusti (71), adapted to a 96-well plate format. To estimate quantity of anthocyanins, sample extracts were made as described for protein assays except with 1% HCl in methanol (v/v) as the extraction solvent. Anthocyanin content estimation is dependent upon differences in absorbances at two distinct wavelengths of each sample incubated with two different pH buffers. Thus, duplicate sample sets of 0.05 mL each were added to 96-well plates. The first set was treated with 0.200 mL of 0.025 M KCl (pH 1.0) and the second set was treated with 0.200 mL of 0.40 M sodium acetate (pH 4.5). Then absorbances were read at both 510 nm and 700 nm for all samples. Using Equations 1 and 2 (71), anthocyanin content was estimated as cyanidin equivalents.

Equation 1: Corrected Absorbance (A) = $(A\ 510 - A\ 700)_{pH\ 1.0} - (A\ 510 - A\ 700)_{pH\ 4.5}$

Equation 2: Anthocyanin content (mg/mL) = [Corrected Absorbance (A) × 449 g mol^{-1} *× [dilution factor] / 26900 L* cm^{-1} mol^{-1}

Terpene Assay

Estimation of total terpenoid content in the targeted plant tissues was based on the protocol from Ghroai (65) and adapted for 96-well plate format. Samples were made as described for protein assays except with 95% methanol in water (v/v) as the extraction solvent. Each sample (0.135 mL) was added to a new microcentrifuge tube along with 1.0 mL of

chloroform and mixed. Concentrated sulfuric acid (0.070 mL) was then added and allowed to react for 1.5 hr. The supernatants were decanted, and the remaining precipitant was resuspended in 1.0 mL of the extraction solvent and mixed thoroughly to dissolve the precipitate. Standards were made from linalool stock solution and the same method was used except with an incubation period of only 5 min. Each standard and sample (0.240 mL) was added to a 96-well plate in triplicate and read at 538 nm.

Alkaloid Assay

Alkaloid content was quantified essentially as described (72). Briefly extracts were made by mixing 0.100 g freeze-dried, ground material and 2 mL methanol. After 24 h of incubation at 20° C, supernatants were drawn off into a clean glass tube, dried under nitrogen (N-vap Model 111, Organomation, West Berlin, MA) and reconstituted with 1 mL of 2N HCl. Contents of the glass tube were then washed successively with chloroform, neutralized with 1N NaOH solution, and 5 mL of phosphate buffer (pH 4.7) and 5 mL Bromocreosol Green were mixed into the neutralized solution. Alkaloids were then extracted using 10 mL chloroform and absorbance was read at $\lambda = 470$ nm in a spectrophotometer.

Lipid Assay

Lipid content was estimated based on total fatty acid content relative to an internal standard (triheptadecanoin) as described (31). Briefly 0.3 mL of toluene containing 2 mg/mL triheptadecanoic acid and 1.0 mL boron trifluoride (14% in methanol) was added to 0.1 g freeze-dried sample in glass screw-capped tubes. After incubation for 1 h at 90° C, 1.5 mL of de-ionized water was added to quench the reaction. Hexanes (2 mL) were added and the samples were vortex mixed. Samples were centrifuged (300 × g, 1 min, 21° C) to separate phases. The top

organic phase was transferred into a clean glass tube. The remaining aqueous phase was extracted two more times with 2 mL hexanes as just described, and all hexanes for a sample were pooled into the same tube. The total organic phase of each sample was dried under nitrogen (N-vap Model 111, Organomation, West Berlin, MA) and reconstituted in hexanes to make a final concentration of 8 mg lipids (estimated based on the expectation of 10 % lipid content in a given tissue) per 1 mL hexanes. Samples were analyzed via gas chromatography (CP-Sil 88 for FAME, 60 m length, 0.25 mm diameter, 0.20 µm filter, Agilent Technologies, Santa Clara, CA) with a final oven temperature of 200° C (Splitless mode) and flow rate of 9.9 mL/min. Lipid estimations were calculated using Equation 3.

*Equation 3: Lipid (mg) = lipid sample peak area / internal standard peak area * internal standard (mg)*

Extract Preparation for Proliferation Assays

T-47D and MDA-MB-231 human breast adenocarcinoma cell lines (kindly provided by Dr. Ramesh Gupta, Department of Pharmacology and Toxicology, University of Louisville) were used to assess effectiveness of phytochemical extracts to inhibit cell proliferation. T-47D is both progesterone receptor (PR) and estrogen receptor (ER) positive (ATCC, https://www.atcc.org/products/all/HTB-133.aspx#characteristics), whereas MDA-MB-231 (ATCC, https://www.atcc.org/products/all/HTB-26.aspx#characteristics) is ER, PR, and human epidermal growth factor receptor 2 (HER2) negative.

Extracts used for the cell proliferation assays were made either in methanol (blood root, alkaloids), 95% methanol (purple redbud, terpenes), or 1% HCl in methanol (lemon balm, phenolics). Approximately 1.5 – 2.5 g of freeze-dried, ground plant material was extracted three times sequentially with volumes of extraction solvent yielding an overall concentration of 100

mg tissue per 1 mL solvent. The samples were sonicated and centrifuged (3000 × *g*, 4° C, 10 min) after each addition of solvent, and the supernatant was drawn off and dried in a pre-weighed screw-cap glass tube under nitrogen (N-vap Model 111, Organomation, West Berlin, MA). The extracts were then reconstituted in 95% ethanol to yield a concentration of 100 mg dry extract per 1 mL of ethanol. The dried bloodroot sample was not fully resuspended in ethanol, so de-ionized water was added to complete the resuspension, altering ethanol content from 95% to 73% and the sample content to 36 mg/mL solvent. Extracts were then filtered (0.2μ filter) and aliquoted into separate, labelled microcentrifuge tubes.

Cell Culture and Proliferation Assays

MDA-MB-231 cells were grown in 175 cm^2 flasks (Corning Inc, Corning, NY) in Liebovitz's L-15 Medium (Thermo-Fischer Scientific, Waltham, MA) supplemented with 10% FBS (Thermo-fisher Scientific, Waltham, MA) and 1% penicillin/streptomycin solution (Thermo-fisher Scientific, Waltham, MA) at 37° C with no CO_2 supplementation. T-47D cells were grown in RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO) supplemented with 10% FBS (Thermo-fisher Scientific, Waltham, MA), 1% penicillin/streptomycin, (Thermo-fisher Scientific, Waltham, MA) and 0.2 units/mL bovine insulin at 37° C with CO_2 supplementation. To dislodge cells, the medium was aspirated, and cells were rinsed with 2 mL 0.25% Trypsin-EDTA (Thermo-fisher Scientific, Waltham, MA). The trypsin was then aspirated, and the flask was incubated at 37° C with an additional 7 mL of 0.25% Trypsin-EDTA. To ensure detachment, cells were viewed under microscope. After detachment, 7 mL of Liebovitz's L-15 medium supplemented with 10% FBS (Thermo-fisher Scientific, Waltham, MA) and 1% penicillin/streptomycin solution (Thermo-fisher Scientific, Waltham, MA) was added, and cells were transferred to a 15 mL centrifuge tube. Cells were pelleted (150 × *g*, 3 min, 23° C), and the

media was aspirated prior resuspension in 1 mL fresh media. The cells were diluted (0.02 mL cells in 1 mL media), and 0.015 mL of diluted cell solution was used to count cells using a hemocytometer and equation 4:

Equation 4: Cell Concentration (cells/µL) = ((number of cells counted/4) x 1x10^4) x 50 [dilution factor]) / 1000 µL

Cells were seeded at 10,000 cells per well in all wells except G12 and H12 on a 96-well plate and allowed to attach overnight (incubated at 37° C). After cell attachment, media was removed by aspiration and 0.1 ml treatment media was added in triplicate for columns 1-3 (lemon balm) 4-6 (purple redbud) 7-9 (bloodroot) and 10-12 (vehicle control). For MDA-MB-231 cell assay, each treatment was diluted to a final concentration of 1 mg/ml in growth media as described in Table 2.

Table 2: Cell proliferation assay treatments for MDA-MB-231. Ethanol was used solely for vehicle control

	Melissa officinalis	*Cercis canadensis*	*Sanguinaria canadensis*	Vehicle Control
Extract or ethanol (mL)	0.012	0.012	0.033	0.024
Media (mL)	1.188	1.188	1.167	1.176
% ethanol	1	1	2	2

For the T-47D cell line, assay protocol was as above, except cells were seeded at 5,000 cells per well and the lemon balm and redbud extracts were diluted to a concentration of 0.5 mg/ml of media and bloodroot to 0.125 mg/mL media prior to being added to row A (Table 3). All treatments were serially diluted by 2-fold from row A to row H then added to the corresponding cells (MDA-MB231 or T-47D). The plate was incubated at 37° C for 72 h.

Table 3: Cell proliferation assay treatments for T-47D. Ethanol was used solely for vehicle control

	Melissa officinalis	*Cercis canadensis*	*Sanguinaria canadensis*	Vehicle Control
Extract or ethanol (mL)	0.025	0.025	0.0174	0.025
Media (mL)	4.975	4.975	4.983	4.975
% ethanol	0.5	0.5	0.25	0.5

After 72 h, cells were subjected to an MTT cell proliferation assay (OZ Biosciences, San Diego, CA) per manufacturer's instructions. The media was aspirated, and the cells were washed with 1× PBS solution (0.1 mL) prewarmed to 37° C. The PBS was then aspirated, and cells were treated with 1× MTT working solution and incubated at 37° C for 2.5 h (T-47D) or 4 h (MDA-MB-231). After incubation, 0.1 mL solubilization solution was added to each well and mixed until crystals fully dissolved. The plate sample absorbance was read at $\lambda = 570$ nm.

Data Analysis

All phytochemical assay data were analyzed using Microsoft Excel. For the protein, starch, terpene, alkaloid, and phenolic assays, a standard curve was formulated and used to estimate concentrations of each phytochemical in the tissue samples. Anthocyanin concentration was estimated utilizing equations 1 and 2. Quantification of lipids was based on retention times of fatty acid methyl ester standards and equation 3. In assays performed in triplicate and/or with multiple technical replications, variance is given as standard deviation. All phytochemical quantities were estimated as mass of phytochemical content (mg or µg) per gram of freeze-dried plant material and represented as bar graphs with standard deviation noted if applicable.

For the cell proliferation assays, each test extract was compared to vehicle control and significance was determined using a two-way ANOVA followed by Dunnett's multiple comparison test by using GraphPad Prism 8. Percent inhibition graphs were made with IC_{50}

values noted based on logarithmic regression. Bar graphs were also made to visually represent percent proliferation of breast cancer cells for the three extracts at each concentration compared to 100% proliferation by vehicle control.

Results and Discussion

Protein

Protein estimates ranged from 2 milligrams protein per gram dry mass to 39 mg/g DM across all samples (Figure 1). Protein quantification results show a relatively high concentration of protein in yarrow flower samples (39 ± 1.4 mg/g dry mass) and dandelion root samples (30 ± 1.5 mg/g). Whereas these two tissues were high in protein content, the flowering tops of St. John's wort yielded only minimal protein content (2.0 ± 0.1 mg/g).

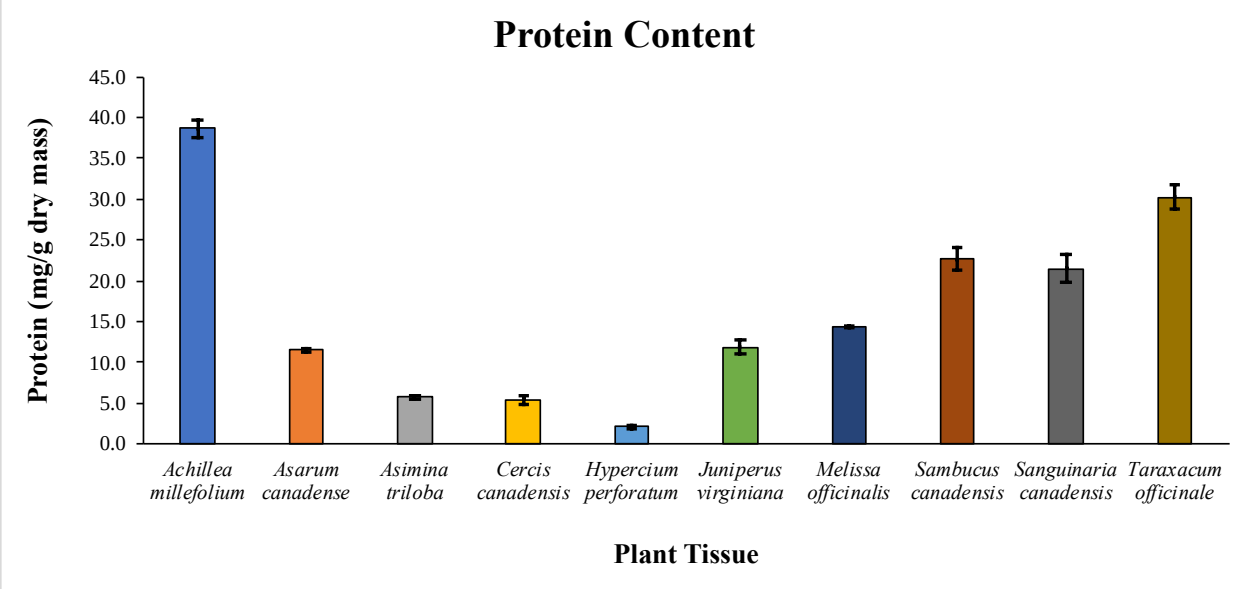


Figure 1: Protein estimation of phytochemicals extracts. Dried powder of each sample was used to make aqueous extracts for protein estimation by Bradford assay. Error bars represent standard deviation of technical replicates.

Starch

Starch content of most samples was found to be approximately 10 mg/g (Figure 2). The highest content was found to be 77 ± 0.9 mg/g in the flowering tops of St. John's wort while the second highest content was found within juniper cones (70 ± 1.8 mg/g). Purple redbud leaves contained less than half of these amounts with only 35 ± 1.0 mg/g.

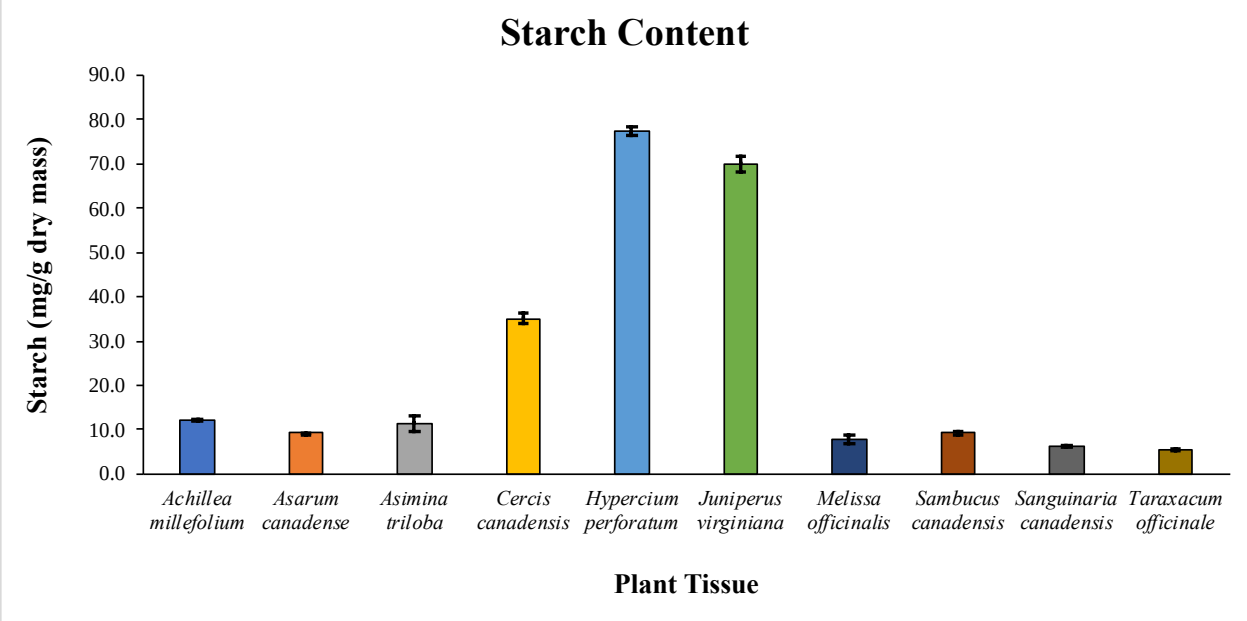


Figure 2: Estimation of starch content in selected plant tissues. Powdered, freeze-dried tissues were extracted with ethanol/water/acetic acid (75/25/1 v/v/v) and tested for starch content using iodine reagent. Starch content is represented by columns, and standard deviation is represented by error bars.

Phenolics

Phenolic content estimation (Figure 3) revealed a relatively high phenolic content within the lemon balm leaf tissue (43 ± 3.0 mg/g), which is reported to contain high amounts of rosmarinic acid (71), and the flowering tops of St. John's wort (28 ± 1.2 mg/g). High phenolic content in St. John's wort could be in part, a measure of hyperforin and adhyperforin content (69). Conversely, the wild ginger rhizome and dandelion roots yielded only minimal phenolic content (3 ± 0.4 mg/g and 4 ± 0.5 mg/g respectively). Dandelion root is known to include several notable phenolic acids including chicoric acid and caffeic acid (66); however, their quantities are relatively low when compared to protein content (30 ± 1.5 mg/g). Pawpaw fruit phenolic content of 8 ± 0.7 mg/g likely includes protocatechuic acid hexoside and various flavonoids (70). Half of the samples yielded less than 10 mg/g of phenolic content (Figure 3).

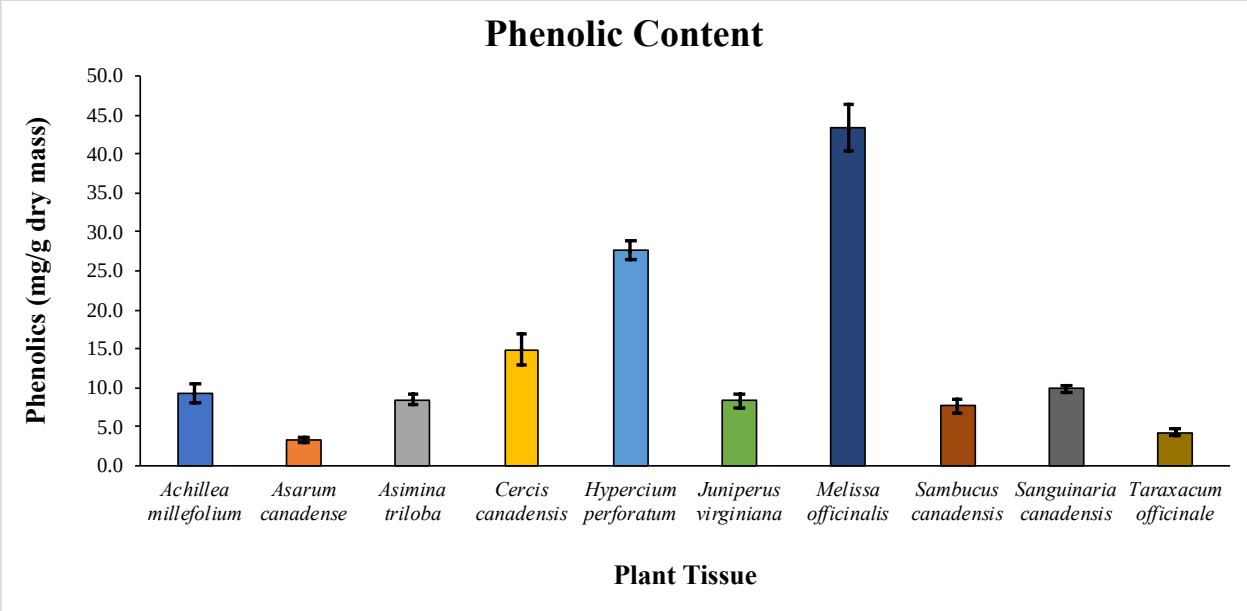


Figure 3: Phenolic content estimations of phytochemical extracts. Quantification was based on the reaction of FC reagent with extracts of freeze-dried plant material in 1% hydrochloric acid in methanol. Bars represent phenolic content, and error bars denote standard deviation among technical replicates.

Anthocyanins

Only four sample tissues yielded results for anthocyanin content (Figure 4), with only one containing a comparatively high quantity. The highest content of anthocyanins was found within elderberry fruits (769 ± 23.1 μg/g). The concentrations of anthocyanins within the yarrow flowers, lemon balm leaves, and purple redbud leaves were minimal in comparison to the elderberry fruits with the second highest concentration found within the leaves of the purple redbud (120 ± 3 μg/g). The findings of anthocyanin content within elderberry and purple redbud tissues are consistent with their reddish-purple colors which often arise in plants from elevated concentration of this subclass of phenolic compounds. Elderberry has been reported to have numerous phenolic compounds including anthocyanidins (68) whose content appears to be approximately 10% of phenolic content (8 ± 0.8 mg/g).

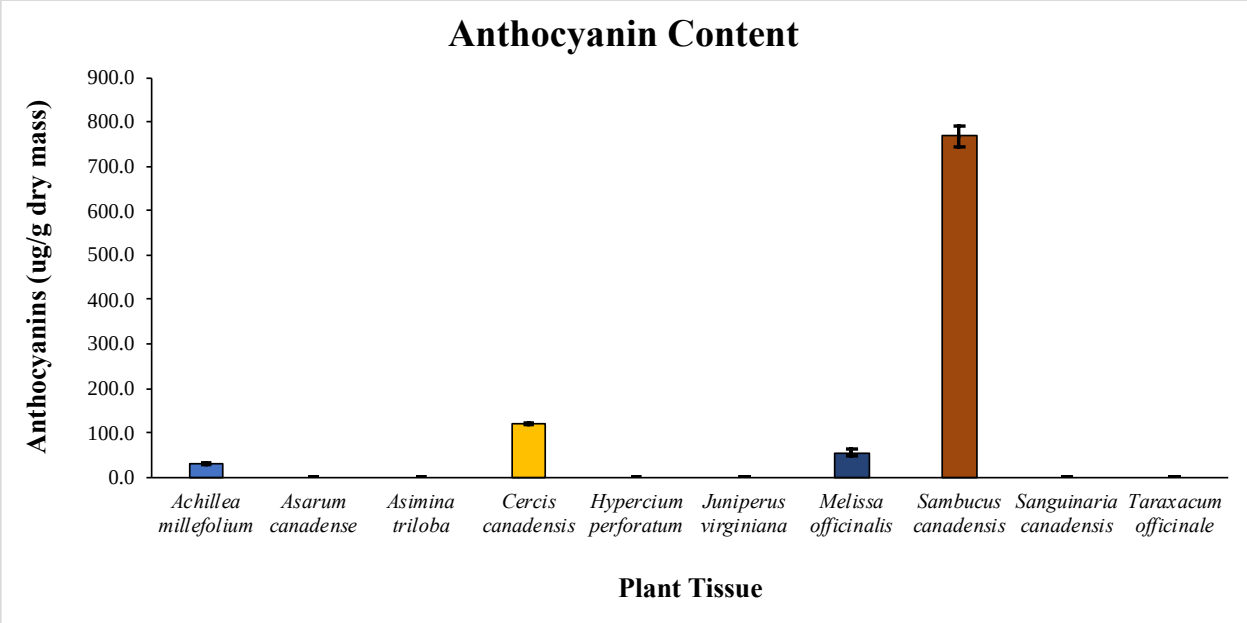


Figure 4: Anthocyanin content estimation in targeted plant tissue extracts. Extracts of freeze-dried, powdered plant material were made in 1% HCl in methanol and tested for anthocyanin content based on colorimetric change of samples at two different pH points tested at two wavelengths. Anthocyanin content is represented by vertical bars, and standard deviation among technical replicates is represented by error bars.

Terpenes

Quantification of terpene content of selected plant tissues (Figure 5) yielded a comparatively high concentration of terpenes within purple redbud leaves (756 ± 28.4 mg/g) and bloodroot roots (589 ± 32.3 mg/g). Pawpaw fruits, dandelion roots, and wild ginger rhizomes, in contrast resulted in lower terpene concentrations with the lowest terpene content being 16 ± 0.6 mg/g (dandelion roots). Terpenes, including sesquiterpene lactones are shown to account for the bitter taste in dandelion leaves (66), but terpenoid content appears to be minimal in dandelion roots. Extract of yarrow flowers yielded a terpenoid content of 274 ± 14.2 mg/g indicative of terpenes such as sabinene, chamazulene, and linalool reported in literature (67). Terpenoids geranial and citronellal (71) are likely present within lemon balm leaf tissue, yielding a total terpene content of 307 ± 29.0 mg/g, similar in value to yarrow flowers and elderberry fruits.

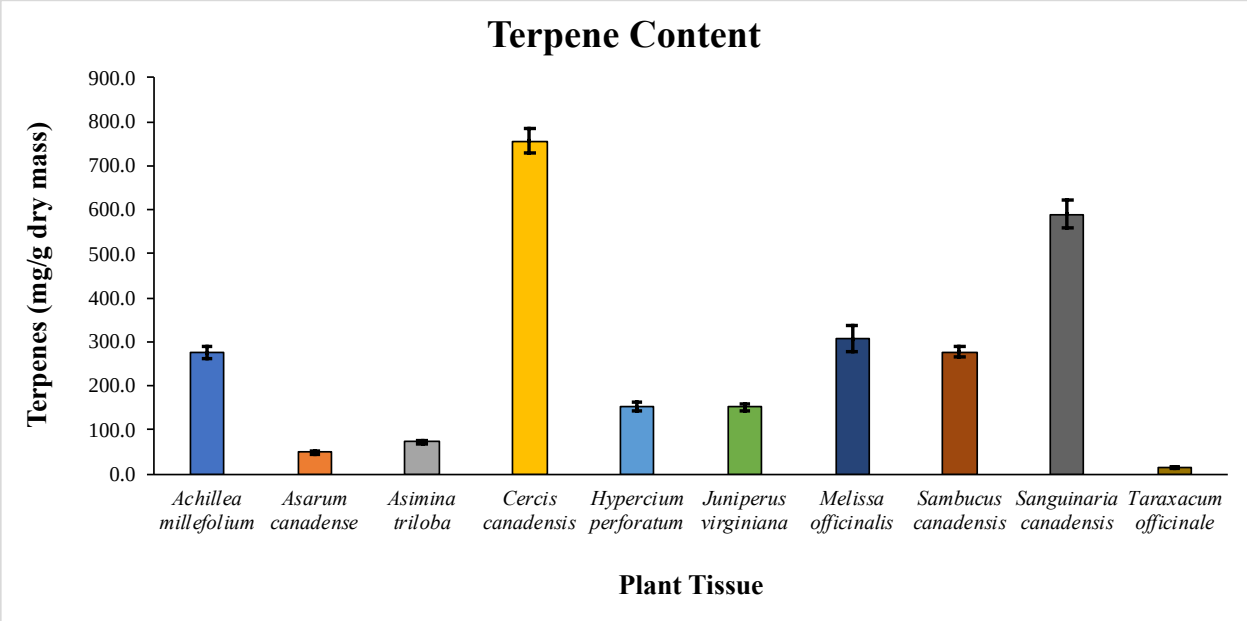


Figure 5: Terpene quantification of phytochemical extracts. Terpene content was estimated using extracts of powdered, dried plant tissues in 95% methanol and based on reaction with sulfuric acid. Error bars denote standard deviation among technical replicates and vertical bars represent terpene content.

Alkaloids

Estimation of alkaloid content within tissue samples (Figure 6) indicated a markedly high concentration of alkaloids in bloodroot roots (65 mg/g) compared to other samples. The second highest concentration of alkaloids was found within the cones of juniper (7 mg/g), and the lowest concentration found was 1 mg/g for yarrow flowers. These data may be indicative of the alkaloid sanguinarine (7), which is found in bloodroot. All tissues tested except for bloodroot resulted in alkaloid contents less than 10 mg/g (Figure 6).

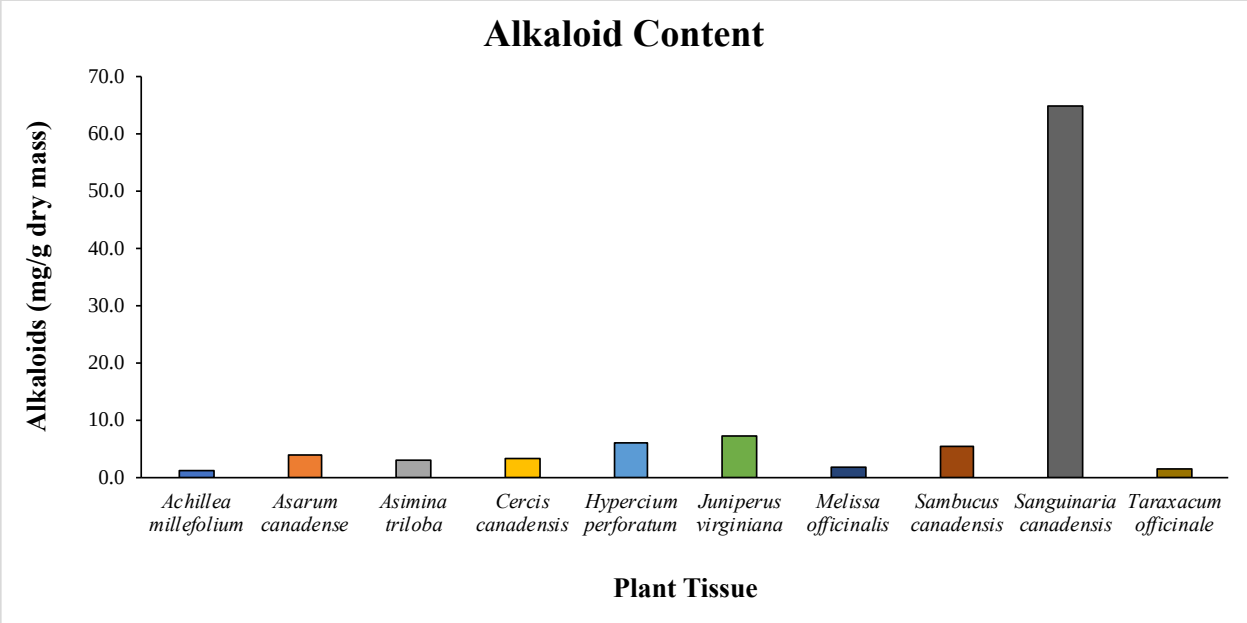


Figure 6: Alkaloid content estimation of targeted plant tissues. Methanolic extracts of powdered, dried plant tissues were made, and alkaloid content was estimated based on the interaction with bromocresol green. Columns represent alkaloid content in targeted plant tissues.

Lipids

Lipid content estimation (Figure 7) resulted with the elderberry fruits having the highest fatty acid methyl esters (71 mg/g) among the 10 selected plants. Wild ginger rhizomes, lemon balm leaves, and redbud leaves yielded similar lipid contents (around 33 mg/g). Dandelion roots and pawpaw fruit contained the least amount of fatty acid methyl esters among the samples (5 mg/g and 4 mg/g, respectively).

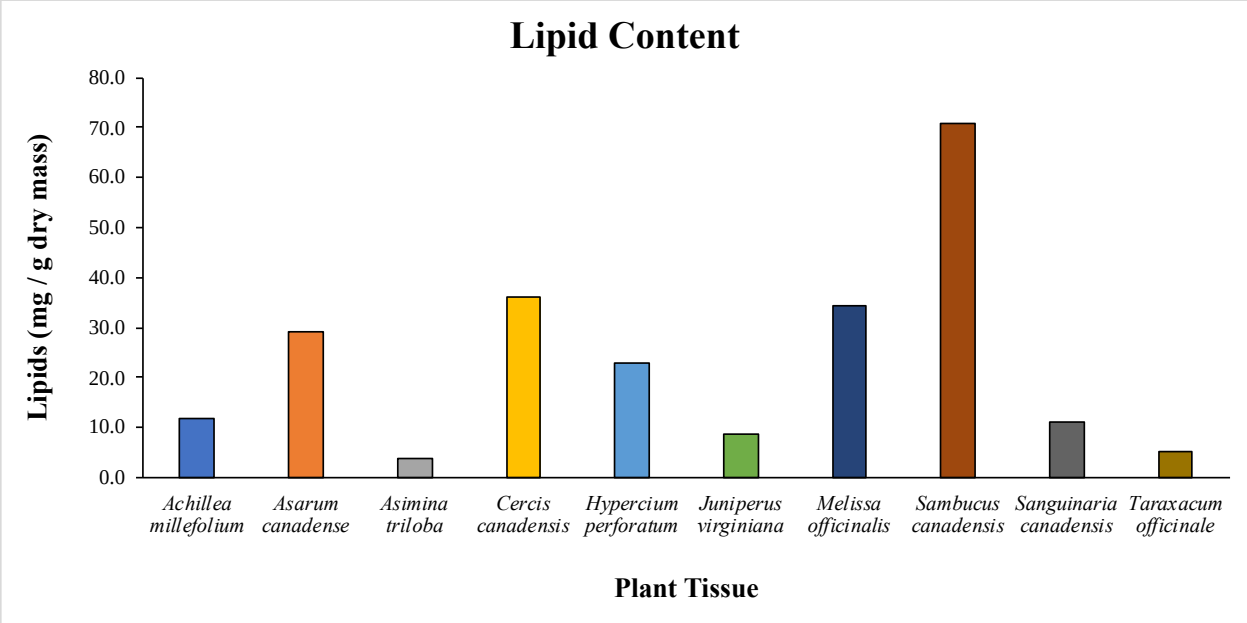


Figure 7: Estimation of lipid content in select native plant tissues. Content of fatty acid methyl esters contained within plant tissues was quantified via GC analysis and based on an internal standard of triheptadecanoic acid. Columns represent fatty acid methyl ester content in selected native plant tissues.

Breast Cancer Cell Proliferation Assays

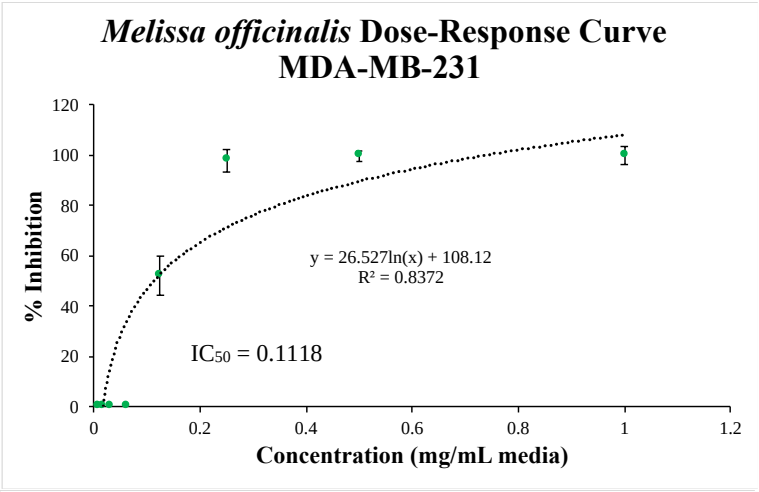
Data from the phytochemical assays were used to formulate a table ranking each of the plant tissues on a scale of 1 to 10 (1 being the highest concentration of indicated phytochemical) for phenolics, terpenes, and alkaloids (Table 3). The three plant tissues with the designation of '1' for phenolics, terpenes, and alkaloids respectively were selected to be used in the cell proliferation assays; these three plant tissues (lemon balm leaves, bloodroot roots, and purple redbud leaves) additionally have some of the overall lowest summed 'scores'. The three phytochemicals selected for comparison (terpenes, alkaloids, and phenolics) were chosen based on the variety of chemical compounds within the respective categories and their potential for anti-cancer activity. These phytochemicals often serve plants as mechanisms for defense and protection against abiotic and biotic stress and, thus, were hypothesized to have potential bioactivity in human cancer cells.

Table 4: Ranking of plant tissues based on phytochemical concentration of phenolics, terpenes, and alkaloids (1=highest concentration; 10=lowest concentration) and the consequent sums of these 3 rankings

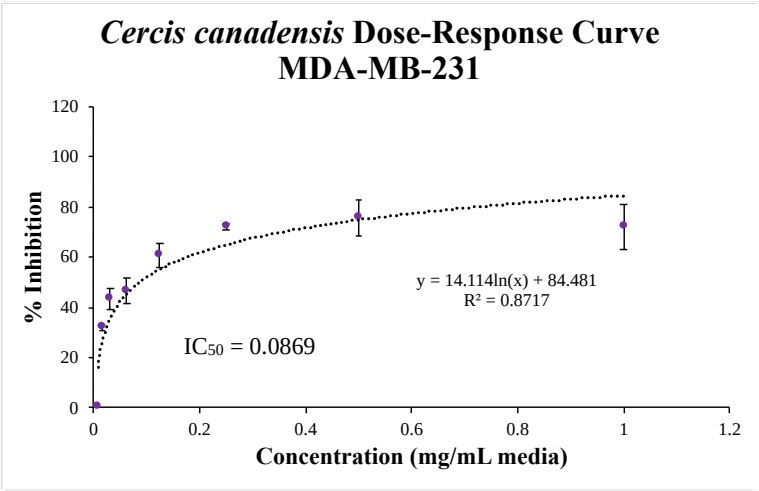
Scientific Name	Common Name	phenolics	terpenes	alkaloids	sum
Achillea millefolium	Yarrow Flower	5	5	10	20
Asarum canadense	Wild Ginger rhizomes	10	9	5	24
Asimina triloba	Pawpaw fruit	6	8	7	21
Cercis canadensis	Purple Redbud leaves	3	1	6	10
Hypercium perforatum	St. John's Wort FT	2	6	3	11
Juniperus communis	Juniper cones	7	7	2	16
Melissa officinales	Lemon Balm leaves	1	3	8	12
Sambucus canadensis	Elderberry fruits	8	4	4	16
Sanguinaria canadensis	Bloodroot roots	4	2	1	7
Taraxacum officinale	Dandelion root	9	10	9	28

When tested against MDA-MB-231 cancer cells, extracts of lemon balm leaves, bloodroot roots, and purple redbud leaves all showed an inhibitory dose-response curve in a logarithmic concentration-dependent manner that was used to calculate IC_{50} values (Figure 8) and showed statistically significant reduction in cell proliferation (Figure 9). Bloodroot extract yielded the lowest IC_{50} value (0.0002 milligrams dry tissue per milliliter media) (Figure 8) and additionally yielded almost complete inhibition (82 ± 3 %) even at a concentration of 31.5 µg/mL media which was statistically significant ($p<0.0001$) when compared to vehicle control and based on a two-way ANOVA and Dunnett's multiple comparisons test (Figure 9). Extract of purple redbud leaves still yielded statistically significant inhibition at low concentrations, including 15.6 µg/mL media (32 ± 1 %) (Figure 9); however, the purple redbud leaves resulted in the least overall inhibition at a 1 mg/mL media concentration (72 ± 9 %) (Figure 9). Lemon balm leaves showed an intermediate level of inhibition overall when compared to extracts of bloodroot roots and purple redbud leaves and had statistically significant ($p=0.05$) inhibition of 52 ± 8 % at a concentration of 125 µg/mL media (Figure 9).

A



B



C

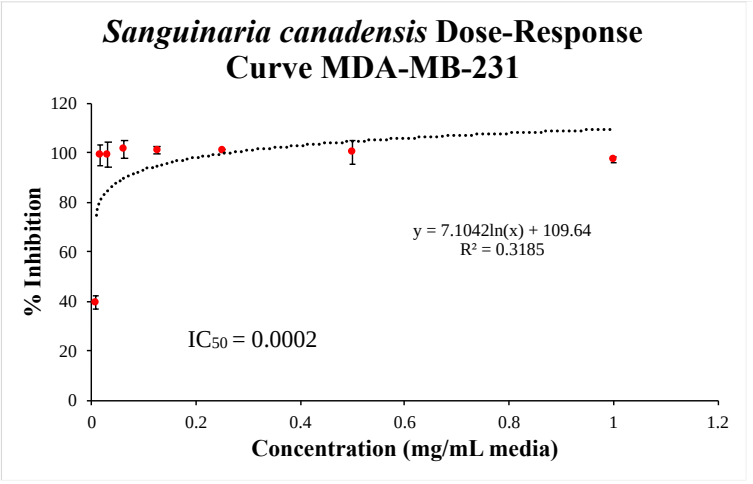
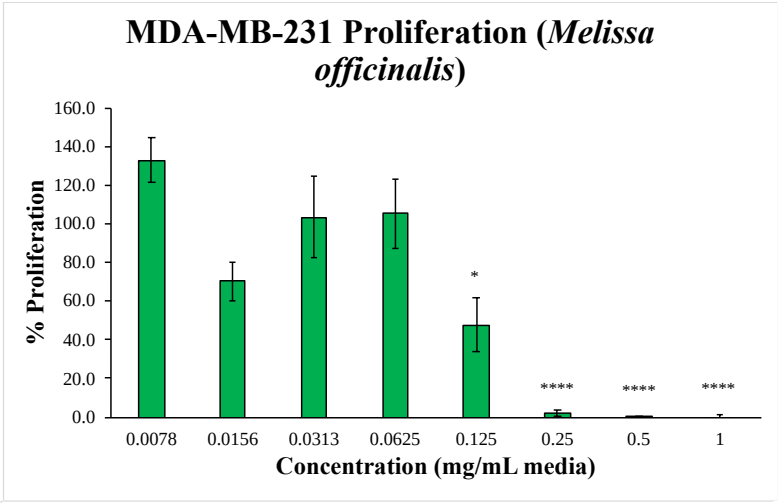
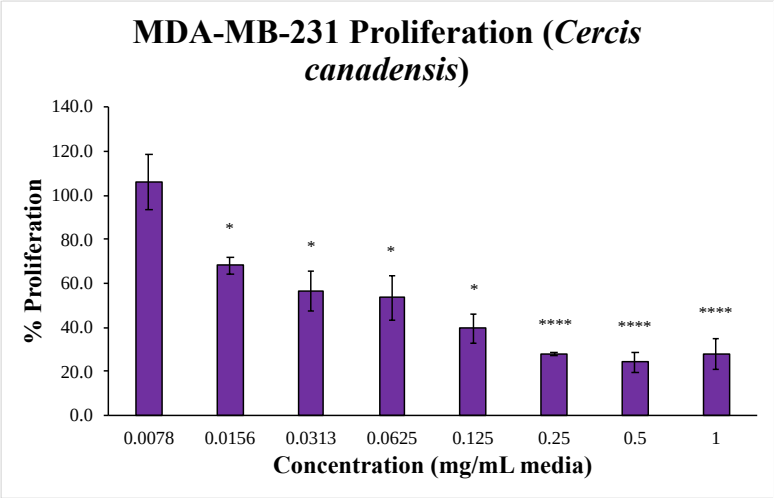


Figure 8: MDA-MB-231 dose-response curves for lemon balm leaves (A), purple redbud leaves (B), and bloodroot roots (C). Percent inhibition of cancer cell growth is indicated at concentrations between 0.0078 mg/mL media and 1 mg/mL media. Standard deviation between technical replicates is represented with error bars and the logarithmic regression equation is noted with R^2 value. IC_{50} values were calculated using the logarithmic equation, and these are noted on the graphs.

A



B



C

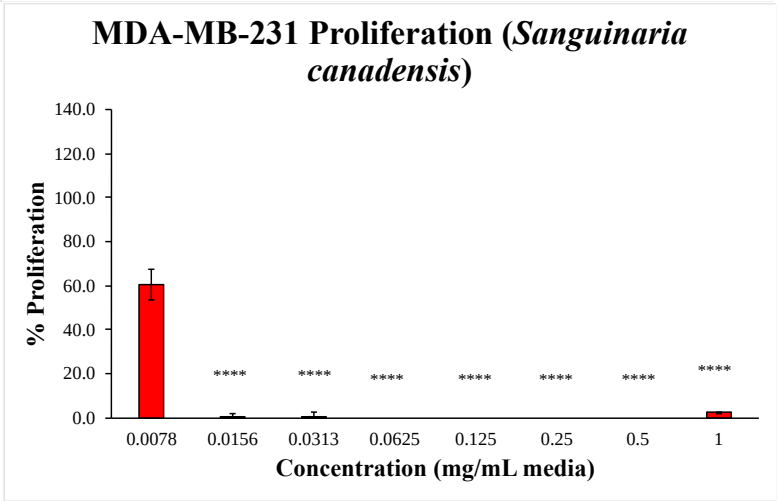


Figure 9: Effects of lemon balm leaves (A), purple redbud leaves (B) and bloodroot roots (C) extracts on proliferation of MDA-MB-231 cancer cells relative to vehicle control (100% proliferation). Significance based on a two-way ANOVA and Dunnett's multiple comparisons test and represented by asterisks (* p=0.05; ** p=0.01; *** p=0.001; **** p <0.0001).

Extracts of lemon balm, bloodroot, and purple redbud yielded a logarithmic, concentration-dependent inhibitory dose-response curve which was used to calculate IC_{50} values (Figure 10) and showed statistically significant reduction in cell proliferation (Figure 11) of the endocrine responsive cell line T-47D. The extract of bloodroot roots again resulted in the lowest IC_{50} value (0.0005 mg/mL media) (Figure 10) compared to extracts of lemon balm leaves (IC_{50} = 0.0416) and purple redbud leaves (IC_{50} = 0.0082). Purple redbud leaves yielded intermediate levels of inhibition at all concentrations yet still resulted in a dose-dependent response (Figure 11) with 98 ± 15% proliferation at a concentration of 3.9 µg/mL media and only 26 ± 18% proliferation at a concentration of 0.5 mg/mL ($p=0.05$). Extract of bloodroot roots yielded statistically significant inhibition (77 ± 14%) at concentrations as low as 7.8 µg/mL media ($p=.01$) and 0% proliferation beginning at 31.3 µg/mL ($p=0.01$). Extract of lemon balm leaves yielded statistically significant ($p=0.05$) inhibition at a concentration of 125 µg/mL media (78 ± 3%) with the highest inhibition at 250 µg/mL media (92 ± 5%).

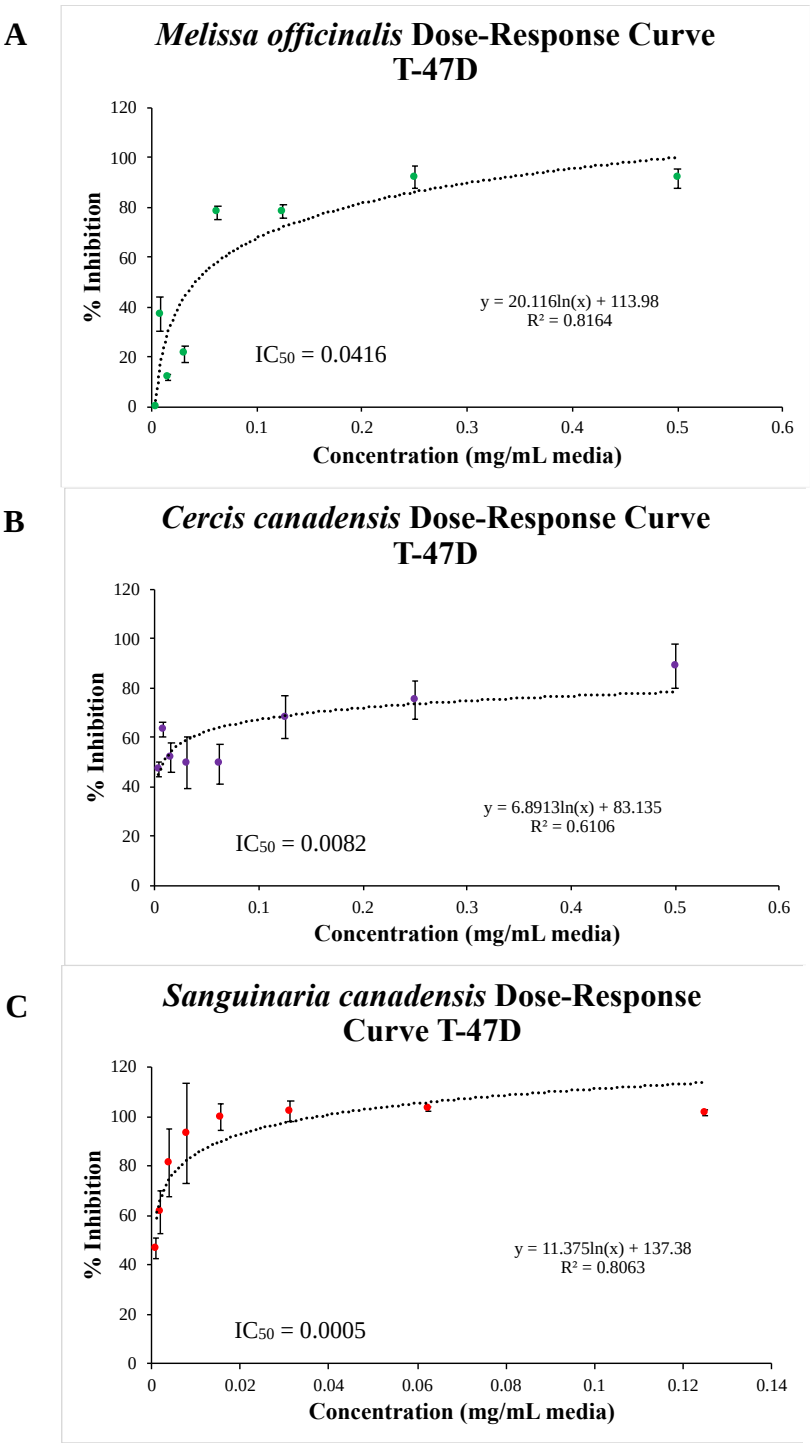


Figure 10: T-47D dose-response curves for lemon balm leaves (A), purple redbud leaves (B), and bloodroot roots (C). Percent inhibition of cancer cell growth is indicated at concentrations between 0.0010 mg/mL media and 0.125 mg/mL media for bloodroot and 0.0039 mg/mL media and 0.5 mg/mL media. Standard deviation between technical replicates is represented with error bars and the logarithmic regression equation is noted with R^2 value. IC_{50} values were calculated using the logarithmic equation, and these are noted on the graphs.

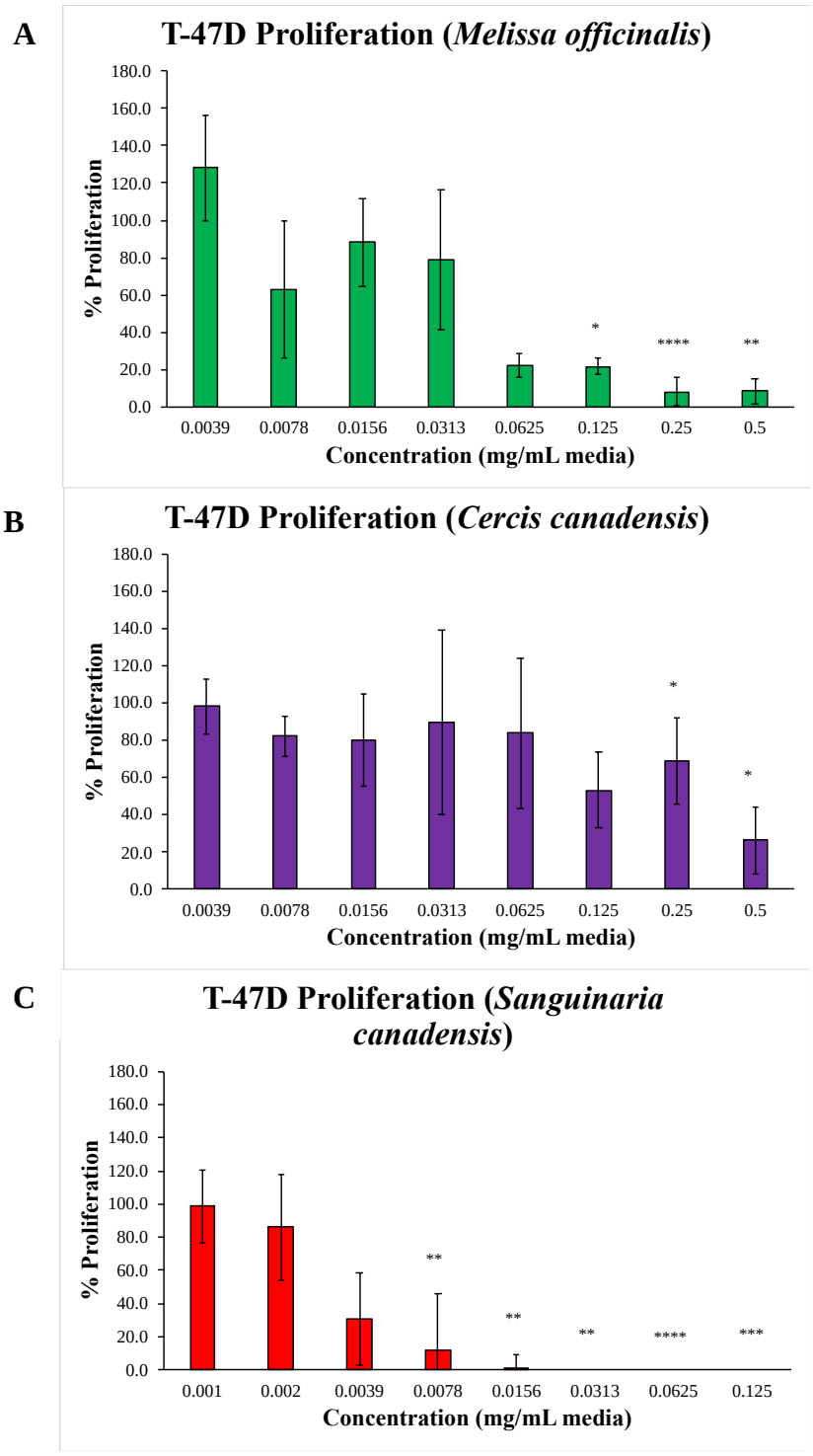


Figure 11: Effects of lemon balm leaves (A), purple redbud leaves (B) and bloodroot roots (C) extracts on proliferation of T-47D cancer cells relative to vehicle control (100% proliferation). Significance based on a two-way ANOVA and Dunnett's multiple comparisons test and represented by asterisks (* p=0.05; ** p=0.01; *** p=0.001; **** p <0.0001).

Conclusion and Future Directions

Phytochemical analysis of extracts made from freeze-dried ground Kentucky native plants showed a diverse range of phytochemical contents including protein, starch, phenolic, anthocyanin, terpene, alkaloid, and lipid contents within these targeted species. The three tissues selected for cell proliferation assays (lemon balm leaves, purple redbud leaves, and bloodroot roots) yielded a concentration-dependent inhibitory dose response for both an endocrine responsive (T-47D) and a triple negative (MDA-MB-231) breast cancer cell line. Extract of bloodroot roots appears to be the most promising for further studies due to its significant reduction in breast cancer cell proliferation, though purple redbud leaves and lemon balm leaves also merit further testing.

Although all extracts resulted in a logarithmic inhibitory response, repetition of the MTT assays is required to create better fitting logarithmic equations. The current dose-response curves likely underestimate the effects on the cancer cell lines. For example, the IC_{50} for bloodroot root extract may be actually lower than the calculated concentration as the logarithmic curve does not fit due to the majority of the concentrations yielding almost full inhibition of cell proliferation of MDA-MB-231 cells. Repetition at lower concentrations would thus provide a better logarithmic fit.

The MTT assay used in this study assesses levels of mitochondrial activity, which was used synonymously with the term ‘cell proliferation.’ This assay has limitations in that mitochondrial activity is not necessarily indicative of the number of viable cells. Thus, further cell proliferation assays are required to better determine the anti-proliferative effects of the three selected extracts. Such possibilities include assays quantifying levels of DNA synthesis and counting live/dead cells based on trypan blue exclusion.

Next steps also include fractionation of the extracts via reverse phase chromatography, analysis of these fractions on HPLC to help identify metabolites present, and repetition of MTT cell assays using the fractions to evaluate bioactivity.

Acknowledgements

I would like to first thank Dr. Schultz for allowing me the opportunity to conduct this research that has allowed me to view scientific understanding in a new lens and have a greater appreciation for plants, biology, and research. I am very thankful for his constant guidance and support and for pushing me to be a better scientist and student. I thank Dr. Menze and Dr. Mueller for taking the time to read through my thesis, attend my defense, and offer valuable feedback. I would also like to thank Dr. Gupta for his kind donation of the breast cancer cell lines used in this study as well as those who started and care for the Korfhage Native Plant Garden from which many of the plants used were collected from. Finally, I would like to thank the members of the Schultz lab: Jared Scott and David Taylor for helping me through the process, taking care of 'my' cells, and answering my numerous questions at any hour of the day; and Pranav Tyagi and Lily Wilding for working alongside me and always lending an ear. Everyone has been extremely supportive in this work and has helped me without ever asking for anything in return. For that, I am extremely honored and appreciative.

References

1. Saleem, S., Muhammad, G., Hussain, M. A., and Bukhari, S. N. A. (2018) A comprehensive review of phytochemical profile, bioactives for pharmaceuticals, and pharmacological attributes of *Azadirachta indica*. Phytother. Res. 32, 1241-1272
2. Balunas, M. J., and Kinghorn, A. D. (2005) Drug discovery from medicinal plants. Life Sci. 78, 431-441
3. Jedrejek, D., Lis, B., Rolnik, A., Stochmal, A., and Olas, B. (2019) Comparative phytochemical, cytotoxicity, antioxidant and haemostatic studies of *Taraxacum officinale* root preparations. Food Chem. Toxicol. 126, 233-247
4. Budzianowski, J. (1997) Coumarins, caffeoyltartaric acids and their artifactual methyl esters from *Taraxacum officinale* leaves. Planta Med. 63, 288-288
5. Ahmadi-Dastgerdi, A., Ezzatpanah, H., Asgary, S., Dokhani, S., and Rahimi, E. (2017) Phytochemical, Antioxidant and Antimicrobial Activity of the Essential Oil from Flowers and Leaves of *Achillea millefolium subsp millefolium*. J. Essent. Oil Bear. Plants. 20, 395-409
6. Tahghighi, A., Maleki-Ravasan, N., Djadid, N. D., Alipour, H., Ahmadvand, R., Karimian, F., and Yousefinejad, S. (2019) GC-MS analysis and anti-mosquito activities of *Juniperus virginiana* essential oil against *Anopheles stephensi* (Diptera: Culicidae). Asian Pac. Trop. Biomed. 9, 168-175
7. Croaker, A., King, G. J., Pyne, J. H., Anoopkumar-Dukie, S., and Liu, L. (2016) *Sanguinaria canadensis*: Traditional Medicine, Phytochemical Composition, Biological Activities and Current Uses. Int. J. Mol. Sci. 17, 32

8. Mathieu, F., Charlebois, D., Charles, M. T., and Chevrier, N. (2015) Biochemical Changes in American Elder (*Sambucus canadensis*) Fruits during Development. I International Symposium on Elderberry 1061, 61-72

9. Lamy, S., Muhire, E., and Annabi, B. (2018) Antiproliferative efficacy of elderberries and elderflowers (*Sambucus canadensis*) on glioma and brain endothelial cells under normoxic and hypoxic conditions. J. Funct. Food. 40, 164-179

10. Weidner, C., Rousseau, M., Plauth, A., Wowro, S. J., Fischer, C., Abdel-Aziz, H., and Sauer, S. (2015) *Melissa officinalis* extract induces apoptosis and inhibits proliferation in colon cancer cells through formation of reactive oxygen species. Phytomedicine 22, 262-270

11. Magalhaes, D. B., Castro, I., Lopes-Rodrigues, V., Pereira, J. M., Barros, L., Ferreira, I., Xavier, C. P. R., and Vasconcelos, M. H. (2018) *Melissa officinalis* L. ethanolic extract inhibits the growth of a lung cancer cell line by interfering with the cell cycle and inducing apoptosis. Food Funct. 9, 3134-3142

12. Nicolaou, K. C., Yang, Z., Liu, J. J., Ueno, H., Nantermet, P. G., Guy, R. K., Claiborne, C. F., Renaud, J., Couladouros, E. A., Paulvannan, K., and Sorensen, E. J. (1994) Total synthesis of taxol. Nature 367, 630-634

13. Osborne, C. K. (1998) Drug therapy - Tamoxifen in the treatment of breast cancer. N. Engl. J. Med. 339, 1609-1618

14. Longacre, M., Snyder, N. A., Housman, G., Leary, M., Lapinska, K., Heerboth, S., Willbanks, A., and Sarkar, S. (2016) A Comparative Analysis of Genetic and Epigenetic Events of Breast and Ovarian Cancer Related to Tumorigenesis. Int. J. Mol. Sci. 17, 17

15. Slamon, D. J., Leyland-Jones, B., Shak, S., Fuchs, H., Paton, V., Bajamonde, A., Fleming, T., Eiermann, W., Wolter, J., Pegram, M., Baselga, J., and Norton, L. (2001) Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N. Engl. J. Med. 344, 783-792

16. Aumsuwan, P., Khan, S. I., Khan, I. A., Avula, B., Walker, L. A., Helferich, W. G., Katzenellenbogen, B. S., and Dasmahapatra, A. K. (2015) Evaluation of wild yam (*Dioscorea villosa*) root extract as a potential epigenetic agent in breast cancer cells. In Vitro Cell. Dev. Biol.-Anim. 51, 59-71

17. Nassan, M. A., Soliman, M. M., Ismail, S. A., and El-Shazly, S. (2018) Effect of *Taraxacum officinale* extract on PI3K/Akt pathway in DMBA-induced breast cancer in albino rats. Biosci. Rep. 38, 11

18. Setzer, W. N. (2018) The Phytochemistry of Cherokee Aromatic Medicinal Plants. Medicines 5, 121

19. Seidlova-Wuttke, D., Thelen, P., and Wuttke, W. (2006) Inhibitory effects of a black cohosh (*Cimicifuga racemosa*) extract on prostate cancer. Planta Med. 72, 521-526

20. Wang, C. Z., Aung, H. H., Bin, Z., Sun, S., Li, X. L., He, H., Xie, J. T., He, T. C., Du, W., and Yuan, C. S. (2008) Chemopreventive Effects of Heat-processed *Panax quinquefolius* Root on Human Breast Cancer Cells. Anticancer Res. 28, 2545-2551

21. Schultz, D.J., Wickramasinghe, N.S., Ivanova, M.M., Isaacs, S.M., Dougherty, S.M., Imbert-Fernandex, Y., Cunningham, A.R., Chen, C and Klinge, C.M. (2010) Anacardic Acid Inhibits Estrogen Receptor α–DNA Binding and Reduces Target Gene Transcription and Breast Cancer Cell Proliferation Mol Cancer Ther. 9, 594-605.

22. Aggarwal, B. B., Bhardwaj, A., Aggarwal, R. S., Seeram, N. P., Shishodia, S., and Takada, Y. (2004) Role of resveratrol in prevention and therapy of cancer: Preclinical and clinical studies. Anticancer Res. 24, 2783-2840

23. Fico, G., Vitalini, S., Colombo, N., and Tome, F. (2006) Hypericum perforatum *L.*, *H maculatum Crantz.*, *H calycinum L.* and *H pulchrum L.*: Phytochemical and morphological studies. Nat. Prod. Commun. 1, 1129-1132

24. Nam, J. S., Jang, H. L., and Rhee, Y. H. (2017) Antioxidant Activities and Phenolic Compounds of Several Tissues of Pawpaw (*Asimina triloba L. Dunal*) Grown in Korea. J. Food Sci. 82, 1827-1833

25. Majrashi, T. A., Zulfiqar, F., Chittiboyina, A. G., Ali, Z., and Khan, I. A. (2019) Isoquinoline alkaloids from *Asimina triloba*. *Nat. Prod. Res.* **33**, 2823-2829

26. Iwashina, T., and Kitajima, J. (2000) Chalcone and flavonol glycosides from *Asarum canadense* (Aristolochiaceae). Phytochemistry 55, 971-974

27. Schaneberg, B. T., Applequist, W. L., and Khan, I. A. (2002) Determination of aristolochic acid I and II in North American species of Asarum and Aristolochia. *Pharmazie* **57**, 686-689

28. Mouhid, L., de Cedron, M. G., Garcia-Carrascosa, E., Reglero, G., Fornari, T., and de Molina, A. R. (2019) Yarrow supercritical extract exerts antitumoral properties by targeting lipid metabolism in pancreatic cancer. *PLoS One* **14**, 21

29. Pirbalouti, A. G., Nekoei, M., Rahimmalek, M., and Malekpoor, F. (2019) Chemical composition and yield of essential oil from lemon balm *(Melissa officinalis L.)* under foliar applications of jasmonic and salicylic acids. Biocatal. Agric. Biotechnol. 19, 5

30. Yaman, T., Uyar, A., Komuroglu, A. U., Keles, O. F., and Yener, Z. Chemopreventive efficacy of juniper berry oil (*Juniperus communis L.*) on azoxymethane-induced colon carcinogenesis in rat. Nutr. Cancer, 14

31. Browse, J., McCourt, P. J., and Somerville, C. R. (1986) Fatty-acid composition of leaf lipids determined after combined digestion and fatty-acid methyl-ester formation from fresh tissue. Anal. Biochem. 152, 141-145

32. Bai, Y. J., Sun, Y., Xie, J., Li, B., Zhang, D. X., Liang, J., Xiao, C. N., Zhong, A. G., Cao, Y. J., and Zheng, X. H. (2020) The asarone-derived phenylpropanoids from the rhizome of *Acorus calamus var. angustatus* Besser. Phytochemistry 170, 9

33. Haghighi, S. R., Asadi, M. H., Akrami, H., and Baghizadeh, A. (2017) Anti-carcinogenic and anti-angiogenic properties of the extracts of *Acorus calamus* on gastric cancer cells. Avicenna J. Phytomedicine 7, 145-156

34. Cheng, J. T., Chen, S. T., Guo, C., Jiao, M. J., Cui, W. J., Wang, S. H., Deng, Z., Chen, C., Chen, S., Zhang, J., and Liu, A. (2018) Triterpenoid Saponins from the Seeds of *Aesculus chinensis* and Their Cytotoxicities. Nat. Product. Bioprospecting 8, 47-56

35. Li, W. J., Tian, H., Li, L., Li, S. H., Yue, W. M., Chen, Z. T., Qi, L., Hu, W. S., Zhu, Y. C., Hao, B., Gao, C., Si, L. B., and Gao, F. (2012) Diallyl trisulfide induces apoptosis and inhibits proliferation of A549 cells in vitro and in vivo. Acta Biochim. Biophys. Sin. 44, 577-583

36. Clement, J. A., Flood, M. J., Bleich, R. M., Willis, T. J., Kelly, R. M., and Schmitt, J. D. (2013) Diterpenoids and acetylenic lipids from *Aralia racemosa*. Biochem. Syst. Ecol. 51, 4-7

37. Thi, N. D., and Hwang, E. S. (2018) Effects of black chokeberry extracts on metastasis and cell-cycle arrest in SK-Hep1 human liver cancer cell line. Asian Pac. Trop. Biomed. 8, 285-291

38. Shafi, G., Hasan, T. N., Syed, N. A., Al-Hazzani, A. A., Alshatwi, A. A., Jyothi, A., and Munshi, A. (2012) *Artemisia absinthium* (AA): a novel potential complementary and alternative medicine for breast cancer. *Mol. Biol. Rep.* **39**, 7373-7379

39. Taleghani, A., Emami, S. A., and Tayarani-Najaran, Z. (2020) Artemisia: a promising plant for the treatment of cancer. *Bioorg. Med. Chem.* **28**, 22

40. Bala, A., Mukherjee, P. K., Braga, F. C., and Matsabisa, M. G. (2018) Comparative inhibition of MCF-7 breast cancer cell growth, invasion and angiogenesis by *Cannabis sativa L.* sourced from sixteen different geographic locations. *S. Afr. J. Bot.* **119**, 154-162

41. Ali, Z., and Khan, I. A. (2008) Alkaloids and saponins from blue cohosh. *Phytochemistry* **69**, 1037-1042

42. Jarry, H., Thelen, P., Christoffel, V., Spengler, B., and Wuttke, W. (2005) *Cimicifuga racemosa* extract BNO 1055 inhibits proliferation of the human prostate cancer cell line LNCaP. Phytomedicine 12, 178-182

43. Bodinet, C., and Freudenstein, J. (2002) Influence of *Cimicifuga racemosa* on the proliferation of estrogen receptor-positive human breast cancer cells. *Breast Cancer Res. Treat.* **76**, 1-10

44. Fruhwirth, G. O., and Hermetter, A. (2007) Seeds and oil of the Styrian oil pumpkin: Components and biological activities. Eur. J. Lipid Sci. Technol. 109, 1128-1140

45. Shebaby, W. N., El-Sibai, M., Bodman-Smith, K., Karam, M. C., Mroueh, M., and Daher, C. F. (2013) The Antioxidant and Anticancer Effects of Wild Carrot Oil Extract. Phytother. Res. 27, 737-744

46. Zaini, R., Clench, M. R., and Le Maitre, C. L. (2011) Bioactive Chemicals from Carrot (*Daucus carota*) Juice Extracts for the Treatment of Leukemia. J. Med. Food 14, 1303-1312

47. Sautour, M., Mitaine-Offer, A. C., and Lacaille-Dubois, M. A. (2007) The Dioscorea genus: a review of bioactive steroid saponins. *Journal of Natural Medicines* **61**, 91-101

48. Tsai, Y. L., Chiu, C. C., Chen, J. Y. F., Chan, K. C., and Lin, S. D. (2012) Cytotoxic effects of *Echinacea purpurea* flower extracts and cichoric acid on human colon cancer cells through induction of apoptosis. *J. Ethnopharmacol.* **143**, 914-919

49. El--Garawani, I., El Nabi, S. H., Nafie, E., and Almeldin, S. (2019) *Foeniculum vulgare* and *Pelargonium graveolens* Essential Oil Mixture Triggers the Cell Cycle Arrest and Apoptosis in MCF-7 Cells. Anti-Cancer Agents Med. Chem. 19, 1103-1113

50. Wodnicka, A., Huzar, E., Dzieciol, M., and Krawczyk, M. (2019) Comparison of the composition and fungicidal activity of essential oils from fennel fruits cultivated in Poland and Egypt. Pol. J. Chem. Technol. 21, 38-42

51. Graca, V. C., Ferreira, I., and Santos, P. F. (2016) Phytochemical composition and biological activities of *Geranium robertianum L.*: A review. *Ind. Crop. Prod.* **87**, 363-378.

52. Engel, R., Gutmann, M., Hartisch, C., Kolodziej, H., and Nahrstedt, A. (1998) Study on the composition of the volatile fraction of *Hamamelis virginiana*. *Planta Med.* **64**, 251-258

53. Sanchez-Tena, S., Fernandez-Cachon, M. L., Carreras, A., Mateos-Martin, M. L., Costoya, N., Moyer, M. P., Nunez, M. J., Torres, J. L., and Cascante, M. (2012) Hamamelitannin from Witch Hazel (*Hamamelis virginiana*) Displays Specific Cytotoxic Activity against Colon Cancer Cells. *J. Nat. Prod.* **75**, 26-33

54. Zanoli, P., and Zavatti, M. (2008) Pharmacognostic and pharmacological profile of *Humulus lupulus L. J. Ethnopharmacol.* **116**, 383-396

55. Leyte-Lugo, M., Britton, E. R., Foil, D. H., Brown, A. R., Todd, D. A., Rivera-Chavez, J., Oberlies, N. H., and Cech, N. B. (2017) Secondary metabolites from the leaves of the medicinal plant goldenseal (*Hydrastis canadensis*). *Phytochem. Lett.* **20**, 54-60

56. Tataranu, L. G., Georgescu, A. M., Buteica, S. A., Silosi, I., Mogosanu, G. D., Purcaru, S. O., Alexandru, O., Stovicek, O. P., Brindusa, C., Dosa, M., Taisescu, C. I., and Dricu, A. (2017) *Ligustrum vulgare* hydroalcoholic extract induces apoptotic cell death in human primary brain tumour cells. *Farmacia* **65**, 766-771

57. Tanna, I., Pandya, P., Harisha, C. R., Shukla, V. J., and Chandola, H. M. (2013) Pharmacognostical and Phytochemical evaluation of Atasi (*Linum usitatissimum L.).* *Indian J. Tradit. Knowl.* **12**, 688-692

58. Felpin, F. X., and Lebreton, J. (2004) History, chemistry and biology of alkaloids from Lobelia inflata. *Tetrahedron* **60**, 10127-10153

59. Descallar, A. L., Nunez, M. P. S., Cabrera, M. L. N., Martin, T. T. B., Obemio, C. D. G., and Lanojan, R. S. (2016) Phytochemical Analysis and Antioxidant Capacity of *Lycopodium clavatum* Linn. from Lake Sebu, South Cotabato, Philippines. in *2nd International Symposium on Applied Chemistry (ISAC)*, Amer Inst Physics, Tangerang, Indonesia

60. Viapiana, A., Struck-Lewicka, W., Konieczynski, P., Wesolowski, M., and Kaliszan, R. (2016) An Approach Based on HPLC-Fingerprint and Chemometrics to Quality Consistency Evaluation of *Matricaria chamomilla L.* Commercial Samples. Front. Plant Sci. 7, 11

61. Magne, Saladin and Clement, 2006, Chemosphere.62: 650-657

62. Marion M. Bradford (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Analytical Biochemistry, **72** (1–2): 248–254

63. Slinkard and Singleton (1977) Total Phenol Analysis: Automation and Comparison with Manual Methods, Am. J. Enology and Viticulture, 28, 49-55

64. Cheng and Breen (1991) Activity of Phenylalanine Ammonia-Lyase (PAL) and Concentrations of Anthocyanins and Phenolics in Developing Strawberry Fruit, J. Amer. Soc. Hort. Sci. 116, 865-869

65. Ghorai, N, Chakraborty, S, Gucchait, S, Kumar Saha, S, and Biswas, S (2012) Estimation of total Terpenoids concentration in plant tissues using a monoterpene, Linalool as standard reagent, Protocol Exchange

66. Gonzalez-Castejon, M., Visioli, F., and Rodriguez-Casado, A. (2012) Diverse biological activities of dandelion. *Nutr. Rev.* **70**, 534-547

67. Orav, A., Arak, E., and Raal, A. (2006) Phytochemical analysis of the essential oil of *Achillea millefolium L.* from various European Countries. *Nat. Prod. Res.* **20**, 1082-1088

68. Johnson, M. C., Tres, M. D., Thomas, A. L., Rottinghaus, G. E., and Greenlief, C. M. (2017) Discriminant Analyses of the Polyphenol Content of American Elderberry Juice

from Multiple Environments Provide Genotype Fingerprint. *J. Agric. Food Chem.* **65**, 4044-4050

69. Heinrich, M., Daniels, R., Stintzing, F. C., and Kammerer, D. R. (2017) Comprehensive phytochemical characterization of St. John's wort (*Hypericum perforatum L*) oil macerates obtained by different extraction protocols via analytical tools applicable in routine control. Pharmazie 72, 131-138

70. Brannan, R. G., Peters, T., and Talcott, S. T. (2015) Phytochemical analysis of ten varieties of pawpaw (*Asimina triloba L. Dunal*) fruit pulp. Food Chem. 168, 656-661

71. Chizzola, R., Lohwasser, U., and Franz, C. (2018) Biodiversity within *Melissa officinalis:* Variability of Bioactive Compounds in a Cultivated Collection. *Molecules* **23**, 13

72. Giusti, M. M. and R. E. Wrolstad (2001). "Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy." Current Protocols in Food Analytical Chemistry - 1.2.1-1.2.13

73. Tabasum, S., Khare, S., and Jain, K. (2016) Spectrophotometric quantification of total phenolic, flavonoid and alkaloid contents of *Abrus precatorius L*. seeds. *Asian J Pharm Clin Res* **9**, 371-374

74. Mattarelli, P., Epifano, F., Minardi, P., Di Vito, M., Modesto, M., Barbanti, L., and Bellardi, M. G. (2017) Chemical Composition and Antimicrobial Activity of Essential Oils from Aerial Parts of *Monarda didyma* and *Monarda fistulosa* Cultivated in Italy. *J. Essent. Oil Bear. Plants.* **20**, 76-86

75. Ashrafi, B., Ramak, P., Ezatpour, B., and Talei, G. R. (2019) Biological activity and chemical composition of the essential oil of *Nepeta cataria L. J. Res. Pharm.* **23**, 336-343

76. Montserrat-de la Paz, S., Fernandez-Arche, M. A., Angel-Martin, M., and Garcia-Gimenez, M. D. (2014) Phytochemical characterization of potential nutraceutical ingredients from Evening Primrose oil (*Oenothera biennis L.*). *Phytochem. Lett.* **8**, 158-162

77. Singh, S., Dubey, V., Singh, D. K., Fatima, K., Ahmad, A., and Luqman, S. (2017) Antiproliferative and antimicrobial efficacy of the compounds isolated from the roots of *Oenothera biennis L.* J. Pharm. Pharmacol. 69, 1230-1243

78. King, M. L., and Murphy, L. L. (2010) Role of cyclin inhibitor protein p21 in the inhibition of HCT116 human colon cancer cell proliferation by American ginseng (*Panax quinquefolius*) and its constituents. *Phytomedicine* **17**, 261-268

79. Dittbrenner, A., Mock, H. P., Borner, A., and Lohwasser, U. (2009) Variability of alkaloid content in *Papaver somniferum L. J. Appl. Bot. Food Qual.-Angew. Bot.* **82**, 103-107

80. Wang, S. J., Wang, X. H., Dai, Y. Y., Ma, M. H., Rahman, K., Nian, H., and Zhang, H. (2019) *Prunella vulgaris*: A Comprehensive Review of Chemical Constituents, Pharmacological Effects and Clinical Applications. *Curr. Pharm. Design* **25**, 359-369

81. Gao, W., Liang, H., Li, Y. L., Liu, Y. Y., and Xu, Y. X. (2019) Root extract of *Prunella vulgaris* inhibits in vitro and in vivo carcinogenesis in MCF-5 human breast carcinoma cells via suppression of angiogenesis, induction of apoptosis, cell cycle arrest and modulation of PI3K/AKT signalling pathway. *J. Buon* **24**, 549-554

82. Marmol, I., Jimenez-Moreno, N., Ancin-Azpilicueta, C., Osada, J., Cerrada, E., and Rodriguez-Yoldi, M. J. (2020) A Combination of *Rosa Canina* Extracts and Gold

Complex Favors Apoptosis of Caco-2 Cells by Increasing Oxidative Stress and Mitochondrial Dysfunction. *Antioxidants* **9**, 12

83. Idris, O. A., Wintola, O. A., and Afolayan, A. J. (2017) Phytochemical and antioxidant activities of *Rumex crispus L.* in treatment of gastrointestinal helminths in Eastern Cape Province, South Africa. *Asian Pac. Trop. Biomed.* **7**, 1071-1078

84. Sumathi, S., Kumar, S. S., and Bai, A. L. G. (2011) Evaluation of Phytochemical Constituents and Antibacterial Activities of *Symphytum officinale L*. *J. Pure Appl. Microbiol.* **5**, 323-328

85. Shahhoseini, R., Azizi, M., Asili, J., Moshtaghi, N., and Samiei, L. (2019) Comprehensive Assessment of Phytochemical Potential of *Tanacetum parthenium* (L.): Phenolic Compounds, Antioxidant Activity, Essential Oil and Parthenolide. *J. Essent. Oil Bear. Plants.* **22**, 614-629

86. Mohammadi, A., Mansoori, B., Baradaran, P. C., Khaze, V., Aghapour, M., Farhadi, M., and Baradaran, B. (2017) *Urtica dioica* Extract Inhibits Proliferation and Induces Apoptosis and Related Gene Expression of Breast Cancer Cells In Vitro and In Vivo. *Clin. Breast Cancer* **17**, 463-470

87. El Haouari, M., and Rosado, J. A. (2019) Phytochemical, Anti-diabetic and Cardiovascular Properties of *Urtica dioica L*. (Urticaceae): A Review. *Mini-Rev. Med. Chem.* **19**, 63-71

88. Zhao, Y. L., Wang, S. F., Li, Y., He, Q. X., Liu, K. C., Yang, Y. P., and Li, X. L. (2011) Isolation of Chemical Constituents from the Aerial Parts of *Verbascum thapsus* and Their Antiangiogenic and Antiproliferative Activities. *Arch. Pharm. Res.* **34**, 703-707

89. Foster, S. and Johnson, R.L. (2006) Desk Reference to Nature's Medicine. *National Geographic*. 12-383.

Appendix

Appendix Table 1: Kentucky native plants, their medicinal uses, and phytochemical content and bioactivity against cancer based on literature. This table was used to narrow down the number of targeted plant tissues to 10.

Scientific Name	Common Name	Tissues Used Medicinally	Months Avaiable for Harvest	Historical Uses (89)	Chemically Active Compounds	Research Indicative of Anti-Cancer Activity/Potential	Availability Rating (1 - 5; easy - hard to obtain)	References
Achillea millefolium	Yarrow	leaves, stems, flowers	June-Nov	helps blood clot; reduce congestion; lower blood pressure; improve circulation	achilleine; salicylic acid; azulenes, flavonoids	activity against pancreatic cancer	1	28, 5
Acorus calamus	Calamus	root	May-July	calm stomach problems; relieve indigestion	asarone; phenylpropanoids	anti-carcinogenic effects on cancer cells	4	32, 33
Aesculus hippocasta	Horse Chestnut	seeds, leaves, flowers, bark	April-June	decrease swelling, fatigue, and tension in legs; improve circulation; for chest pain and rheumatism	triterpene saponins; aescin	cytotoxic effects on MCF-7 cancer cells	4	34
Allium sativum	Garlic	bulb	July-Sept	lowers blood cholesterol; antioxidant; anti-cancer; treat viral and bacterial infections; lower blood sugar levels	allicin; diallyl trisulfide	cell cycle inhibition as well as apoptosis in lung cancer cell lines	1	35
Amelanchier arborea	Downy Serviceberry	berries	March-June	anti-diarrhea; for intestinal worms; anti-oxidant; for consumption	anthocyanins		1	
Aralia racemosa	Spikenard	roots	June-Aug	for skin and ear problems	diterpeoids; acetylenic lipids		3	36
Aronia melanocarpa	Black Chokeberry	stems, leaves, berries	May	anti-cancer; anti-inflammatory; for consumption	polyphenols	inhibits SK-Hep1 cell growth in proportion to concentration	2	37
Artemisia absinthiun	Wormwood	leaves, stems, flowers	July-Sept	get rid of intestinal problems and worms; stimulte appetite, stomach acid, and bile	sesquiterpene lactones - absinthin, anabsinthin, and artabsin; thujone; azulenes	anti-proliferative effects on human breast cancer cells	2	38
Artemisia annua	Sweet Wormword	leaves	Aug-Nov	reduce fever; relieve headaches, dizziness, nosebleeds, and malaria	flavanoids; artemisinin	therapeutic agent against cancer proliferation	2	39
Cannabis sativa	Hemp	flowering tops, leaves, seeds	June-Sept	relieve nausea and pain in chemotherapy; treat malaria, migraines, and menstrual pain	tetrahydrocannabinol; cannabidiol	effect on MCF-7 breast cancer cell growth	1	40

Appendix Table 1: Kentucky native plants, their medicinal uses, and phytochemical content and bioactivity against cancer based on literature. This table was used to narrow down the number of targeted plant tissues to 10.

Scientific Name	Common Name	Tissues Used Medicinally	Months Avaiable for Harvest	Historical Uses (89)	Chemically Active Compounds	Research Indicative of Anti-Cancer Activity/Potential	Availability Rating (1 - 5; easy - hard to obtain)	References
Caulophyllum thalict	Blue Cohosh	root	April-May	induce labor; reduce uterine inflammation; treat kidey infections and arthritis	caulosaponin; alkaloids		5	41
Cichorum intybus	Chicory	leaves, roots	June-Oct	for indigestion; diuretic; clean blood	inulin		2	
Cimicifuga racemosa	Black Cohosh	roots, rhizomes	June-Aug	treat malaria, menstrual irregularities, arthritis, and rheumatism; ease symptoms of menopause		inhibits prostate cancer cell growth in mice; induces apoptosis in estrogen-receptor positive breast cancer cells	1	43, 42
Cucurbita pepo	Pumpkin	seeds, flesh	July-Oct	get rid of intestinal worms; relieve BPH	sterols, cucerbitin, polyphenols		1	44
Daucus carota	Queen Anne's Lace	roots, seeds, leaves, stems	May-Oct	for bladder and kidney conditions and indigestion	alpha and beta carotene; polyacetylenes	anti-cancer activity against human colon cancer cells and breast cancer cell lines MDA-MB-231 and MCF-7; induce apoptosis in leukemia cell lines	4	45, 46
Dioscorea villosa	Wild Yam	roots; tuber	May-June	relieve labor pain and menstrual cramps; anti-inflammatory; dilate blood vessels; make steroid hormones	steroid saponins	reduce proliferation of MDA-MB-231 breast cancer cells	3	47, 16
Echinacea purpurea	Echinacea	whole plant	June-Oct	treat colds and flus; anti-inflammatory	echinacin; cichoric acid	inhibits cell proliferation of colon cancer cells and induces apoptosis	1	48
Foeniculum vulgare	Fennel	seeds	June-Aug	eyewash; for bloating and indigestion; treat cramps and PMS; increase breast milk production	anethole; fenchone; estragole	inhibitor of growth for breast cancer cell lines MCF-7 and MDA MB 237 in time-dependent and conc-dependent manners	1	49, 50
Gaultheria procumbe	Wintergreen	leaves	July-Aug	improve breathing; for aches, pains, and diarrhea	methyl salicylate		3	
Geranium robertianu	Herb-Robert	leaves, stems, roots	May-Sept	diuretic; coagulant; treat diarrhea; help IBS and heavy period flow	tannins; geraniin; flavonoids; phenolics		3	51
Hamamelis virginian	Witch Hazel	leaves, bark, twigs	Oct-Nov	for mouth irritations, dysentery, internal bleeding, and fevers; for minor flesh injuries; in eye drops	flavonoids; hamamelitannin; sesquiterpenes; phenylpropanoids	reduces viability and induces apoptosis of colon cancer cells	1	52, 53
Caulophyllum thalict	Blue Cohosh	root	April-May	induce labor; reduce uterine inflammation; treat kidey infections and arthritis	caulosaponin; alkaloids		1	41

Appendix Table 1: Kentucky native plants, their medicinal uses, and phytochemical content and bioactivity against cancer based on literature. This table was used to narrow down the number of targeted plant tissues to 10.

Scientific Name	Common Name	Tissues Used Medicinally	Months Avaiable for Harvest	Historical Uses (89)	Chemically Active Compounds	Research Indicative of Anti-Cancer Activity/Potential	Availability Rating (1 - 5; easy - hard to obtain)	References
Hydrastis canadensis	Goldenseal	rhizomes, stems	April-May	fight intestinal worms and parasites; treat diarrhea; anti-cancer	berberine; hydrastine; alkaloids; flavonoids		3	55
Hypercium prolificum	St. John's Wort	dried flowering tops	year round	treat depression; for heart problems, jaundice, bruises, and hysteria; treat anxiety and insomnia; antitumor activity	tannins; flavonoids; hypericin; hyperforin; triterpenes		1	23
Juniperus communis	Juniper	cones	all year	treat urinary tract infections; antioxidant; treat snakebites; diuretic	flavonoids, tannins; juniperin	anti-cancer effects against colon cancer	1	6, 30
Ligustrum vulgare	Common Privet	leaves, berries	June-July	prevent bone marrow loss in chemotherapy; treat Parkinson's; treats hyperglycemia	ligustrin	induced apoptosis in brain cancer cells	3	56
Linum usitatissimum	Flax	seed, oil	June-Aug	treats aches and pains; soothes skin irritations; reduces inflammation; boost immune system	alpha-linolenic acid; lignans		1	57
Lobelia inflata	Lobelia	leaves, stems, flowering tops	July-Oct	makes breathing easier; muscle relaxer; treat fevers	alkaloids; lobeline		2	58
Lycopodium clavatum	Clubmoss	whole plant, spores	all year	relieve skin issues; laxative and purgative; treat Alzheimer's	huperzine; alkaloids		2	59
Matricaria recutita	Chamomile	dried flowr heads, oil	July-Sept	for IBS and other stomach issues; helps to fall asleep; treats skin inflammations; inhibit growth of H. pylori	chamazulene; phenolic acids		1	60
Medicago sativa	Alfalfa	stems, leaves	June-Sept	treat anemia, diabetes, and indigestion; increase urination; increase breast milk production; lower cholesterol	saponins		1	
Melissa officinalis	Lemon Balm	leaf	June-Aug	soothe bites and stings; treat cold sores; for stress and anxiety; used for Alzheimer's	citral; citronellal; jasmonic and salicylic acid	anti-cancer; induces apoptosis in lung and colon cancers	1	29, 10, 11
Mentha x piperita	Peppermint	whole plant, leaves, oil	June-Oct	treat indigestion, flatulence, nausea, and vomiting; inhibit smooth muscle contraction; help with GERD and IBS	menthol		1	
Mitchella repens	Partridge Berry	whole plant	May-July	ease childbirth; calm nervous system; ease digestion			4	

Appendix Table 1: Kentucky native plants, their medicinal uses, and phytochemical content and bioactivity against cancer based on literature. This table was used to narrow down the number of targeted plant tissues to 10.

Scientific Name	Common Name	Tissues Used Medicinally	Months Avaiable for Harvest	Historical Uses (89)	Chemically Active Compounds	Research Indicative of Anti-Cancer Activity/Potential	Availability Rating (1 - 5; easy - hard to obtain)	References
Monarda didyma	Bee Balm	leaves	July-Sept	cold remedy; for stomach pain; treat fevers, nausea, and sore throats; oral antibiotic	thymol		1	74
Nepeta cataria	Catnip	leaves, flowers	July-Oct	stimulates sweating; for coughs, colds, and flus; for stomach pain; treat ADHD	nepatalactone		3	75
Nicotiana rustica	Tobacco	leaves	June-Oct	stimulates nervous system; carcinogenic	nicotine		1	
Oenothera biennis	Evening primrose	seeds, oil, leaves	July-Oct	may help with breast cancer; for rheumatoid arthritis; prevent diabetic nerve degeneration	gamma linolenic acid, campesterol	antiproliferative effects on several cancer cell lines	3	76, 77
Panax quinquefolius	American Ginseng	root	May-June	treat diabetes and cancer; boost immune system	saponins; ginsenosides	causes cell cycle arrest in colon cancer cells	5	78
Papaver somniferum	Opium Poppy	fruits, seeds	May-Aug	blocks nerve impulses for muscle contraction; induces euphoria; relieves pain	alkaloids; morphine; codeine; papaverine		5	79
Prunella vulgaris	Heall All	flowering stems	May-Sept	diuretic; mouthwash; for convulsions and intestinal worms; reduce headaches, fevers, and high blood pressure; antioxidant; anti-cancer	coumarins, phenylpropanoids, triterpenoids, sterols, flavonoids	prevent progression of breast cancer; induces apoptosis	4	80, 81
Prunus serotina	Black Cherry	bark	April-May	for coughs and colds; reduce fever; ease childbirth; in cough syrups			1	
Rosa canina	Dog Rose	fruits, seeds,petals, oil	May-July	for irritated skin and sores; treat diarrhea and coughs	flavonoids; tannins; pectin; citronellal	induces apoptosis in colorectal cancer cells	1	82
Rumex crispus	Yellow Dock	root	May-June	for skin, liver, and respiratory problems; laxative; treat anemia and IBS	anthraquinones; glycosides; tannins; oxalates	induces of apoptosis	3	
Salix alba	White Willow	bark	March-May	reduce fever and inflammation; alleviate pain	alkaloids; saponins, flavonoids		3	83
Sambucus canadens	American Black Elderberry	berries	June-July	boost immune system; for colds and flus	anthocyanins; rutin	antiproliferative effects; induces apoptosis in brain cancer cells	1	8, 9

Appendix Table 1: Kentucky native plants, their medicinal uses, and phytochemical content and bioactivity against cancer based on literature. This table was used to narrow down the number of targeted plant tissues to 10.

Scientific Name	Common Name	Tissues Used Medicinally	Months Avaiable for Harvest	Historical Uses (89)	Chemically Active Compounds	Research Indicative of Anti-Cancer Activity/Potential	Availability Rating (1 - 5; easy - hard to obtain)	References
Sanguinaria canadensis	Bloodroot	rhizomes	March-April	treat sore throat, fevers, and rheumatism; treat tumors; treat spasms and lung issues	alkaloids; sanguinarine; benzophenanthridine		1	7
Sassafrass albidium	Sassafrass	leaves, roots	April-May	heal wounds; good for blood; treat insect bites and reactions from poisonous plants	safrole		2	
Scutellaria lateriflora	Skullcap	dried aerial parts	July-Sept	promote menstruation; calm nervous system; cure rabies; for anorexia, Tourette's, and chronic headaches; sedative; improve bloodflow	scutellarin		3	
Symphytum officinale	Comfrey	leaves, roots	June-Aug	good for internal bleeding and stomach problems	flvonoids, saponins, triterpenoids; phenolics		3	84
Tanacetum parthenium	Feverfew	leaves, flowers	June-Sept	digestive aid; for headaches and migraines; inhibit smooth muscle contractions	camphor; parthenolide		4	85
Taraxacum officinale	Dandelion	leaves, flowers, roots	Feb-Dec	for breast and lung tumors	hydroxycinnamic acids, sesquiterpene lactones, triterpenes, coumarins, phenolic acids, flavonoids	suppresses breast cancer growth and proliferation	1	3, 4, 17
Urtica dioica	Stinging Nettle	leaves, roots	June-Sept	counterirritant; rheumatism remedy	polyphenols, flavonoids, lectins, sterols	inhibits proliferation of breast cancer cells	2	86, 87
Verbascum thapsus	Mullein	leaves, flowers	June-Sept	for coughs and colds; for rashes and tumors; promote sweating; diuretic	verbathasin A; ajugol	anticancer effects against lung cancer	4	88