BMC Complementary Medicine and Therapies

RESEARCH

Open Access

Antiproliferative potential of sweetpotato in breast (BT549) and lung (A549) cancer cell lines

Sochinwechi Nwosisi[1], Dilip Nandwani[1*] and Elbert Lewis Myles[2]

Abstract

Background Researchers have searched for plant-derived medicines over the last few decades. Although much of the focus has been on medicinal herbs, many vegetables, such as sweetpotato, have also been discovered to have medicinal properties due to their high levels of nutrients and phytochemicals. This study aimed to determine the effects of methanolic extracts from sweetpotato leaves and leaves/stems on human lung (A549) and breast (BT549) cancer cell lines. The authors obtained the leafy greens from Tennessee State University's Organic research farm, extracted with methanol and studied for their cytotoxicity.

Methods Alamar blue Assay was used to determine whether the methanolic extracts from fresh sweetpotato leaves (cv. All Purple) and leaves/stems (cv. Carolina Ruby) had the potential to affect cell proliferation in the human lung (A549) and breast (BT549) cell lines in-vitro. Tamoxifen was used as the positive control, while DMSO was used as the negative control.

Results Sweetpotato leaves of the All-Purple cultivar and the stem/leaves of the Carolina Ruby cultivar inhibited lung and breast cancer cell growth in a dose-dependent manner. The All purple sweetpotato produced EC_{50} values of 0.013 µg/µl (R2 = 0.67, $P < 0.05$) and 0.002 µg/µl (R2 = 0.89, $P < 0.05$) in the A549 and BT549 cell lines respectively, using the Alamar blue assay. The BT549 cell line treated with all purple leaf extract was less significant than that of the A549 cell line. However, the sweetpotato stem and leaf extract of Carolina ruby had a more significant cytotoxic effect on the A549 cell line with an EC_{50} value of 0.0014 µg/µl (R2 = 0.99, $P < 0.05$).

Conclusions Anticancer activities of these extracts showed their ability to inhibit the growth of cancer cell lines, such as BT549 (breast cancer) and A549 (lung cancer), in a concentration-dependent manner. Further studies would help determine the bioactive compounds present in these compounds that produce this effect, but more studies would also help determine whether the extracts could induce apoptosis in BT549 and A549 cancer cells, the mechanism of action, and cell cycle progression.

Keywords Anticancer, Cytotoxic activity, Ethnomedicine, In-vitro

Background

Sweetpotato (*Ipomoea batatas*) (L.) Lam is an edible vegetable that is sweet and highly nutritious. It belongs to the Convolvulaceae. The plant is native to tropical regions of America. However, it is widely grown in over 100 countries, primarily in developing nations that are responsible for approximately 95% of the global output [1].

*Correspondence:
Dilip Nandwani
dnandwan@tnstate.edu
[1] Department of Agricultural and Environmental Sciences, Tennessee State University, 3500 John A. Merritt Blvd, Nashville, TN 37209, USA
[2] Department of Biological Sciences, Tennessee State University, 3500 John A. Merritt Blvd, Nashville, TN 37209, USA

A few of the common terms used to refer to the root vegetable in various parts of the world include caiapo (South America), nyami which means "to eat" (West Africa), yam (United States and Canada), kumara (New Zealand), and camote (Latin America) [2] amongst others. It grows as a dicotyledonous, herbaceous perennial vine bearing alternate heart-shaped or palmately lobed leaves and medium-sized sympetalous flowers [3]. From sweetpotato, natural products can be developed for use as medicine, industrial products, and other valuable products [3]. As the crop is not a commonly consumed leafy green vegetable, (it is grown for its large tuberous root) 95–98% of its leaves are thrown away, and the remainder are used to feed animals [4]. Sweet potato leaves (SPL) are mainly consumed in the Pacific Ocean, Africa, Asia, China, Taiwan [5, 6], the Caribbean, America (Southern and Central) and little is consumed in the United States. Sweetpotato is an important summer food crop in southern U.S.A. High in dietary fiber, proteins, iron, calcium, vitamin B, ß-carotene, and zinc, the crop, also has greater tolerance for high moisture, diseases and pests when compared than many other tropical leafy green vegetables [7].

Recent studies have highlighted the importance of diet, particularly the intake of vegetables and fruits, in suppressing disease. In recent years, phytochemicals in plants have attracted significant attention from research communities, food industries, and consumers [8]. Sweetpotato leaves, stems, stalks, peel, and flesh are important sources of bioactive flavonoids, proteins, phenolic acids, carbohydrates, lipids, carotenoids, and anthocyanins [9]. These biologically active substances are responsible for several antioxidant, antidiabetic, anticancer, hepatoprotective, antimicrobial, antiulcer and immunostimulant activities [9]. Sweetpotato leafy vegetable (SPLV) contains bioactive compounds that reduce the characteristic risks for chronic diseases [6]. Research has shown that the leaves of this root crop are a great source of antioxidants and polyphenolics, among which anthocyanins and phenolics are superior in quality to those of other commercial vegetables. Several studies have shown that bioactive compounds in leaves, such as antioxidants, vitamin C, and lutein can help protect against free radicals and prevent diseases connected to oxidation such as cancer, allergies, aging, HIV, cardiovascular problems, age-related macular degeneration, and cataracts [10, 11]. Using a fusion of traditional and biomedical methods SPL have been used by the Akan ethnic group of Ghana to treat diabetes [12]. The ability of antioxidants and other bioactive compounds within the plant's leaves to minimize the risks associated with disease development is due to their ability to promote more favorable antioxidant status, increase free radical scavenging capacity, and thwart processes involved in disease pathogenesis [6]. Furthermore, the polyphenolic content in the leaves, which is greater than that of many other commercially available leafy greens, has various kinds of physiological characteristics; radical scavenging activity; and antimutagenic, antibacterial, anticancer, antidiabetic, and anti-inflammatory effects within and outside the laboratory environments that can benefit human health [10, 11]. In a study by Ghasemzadeh [4], the antioxidant activity of SPL determined by phytochemical analysis was strongly correlated with the total polyphenolic content. Phenolic acids such as monocaffeoylquinic (chlorogenic acid), dicaffeoylquinic, caffeic, and tricaffeoylquinic acids have been reported to be the main phenolic compounds isolated from these greens that restrict cancer cell development [5, 13]. Additional bioactive compounds in the leaves include minerals, sesquiterpenoids, vitamins, anthocyanins, alkaloids, tannins, flavonoids, glycosides, saponins, and enzymes [14]. Leafy green extracts of the sweetpotato have been shown to inhibit the invasion of African American breast cancer cells (MDA-MB 468) by 25.3% according to the Matrigel invasion assay [15]. By investigating the link between the consumption of common local foods and the risk of cancer in Taiwan, using a food frequency questionnaire, researchers discovered a decrease in the risk of lung cancer with increased consumption of vitamin A, α-carotene, and β-carotene in 13 foods, including SPL due to bioactive components present in the leaves [16]. In studying the antimutagenic potential of the methanolic extract from the leaves of *Ipomea batatas* on human stomach cancer and rat liver epithelial cells with the *umu* and *S. typhimurium* Ames' tests, it was concluded that the sweetpotato leafy greens have antimutagenic and anticarcinogenic activity in vitro [17]. Karna et al. [18] as reported by Dhianawaty [19] measured the volume of tumors using noninvasive real-time bioluminescent imaging and discovered that the leafy green extracts inhibited the growth of prostate cancer by 65% in nude mice when administered orally at 400 mg/kg. Karna et al. [18] also discovered that these extracts prevented the proliferation and induction of apoptosis in prostate cancer cells both in vivo and in vitro, suggesting that the leafy greens are nontoxic to tissues in the gut and bone marrow which divide rapidly. The anticancer activity was due to the high polyphenol content in the extract. According to numerous studies, extracts from different sweet potato parts exhibit anticancer and antitumor properties.

For many years, the whole sweetpotato plant (aqueous and ethanolic extracts of leaves, roots and peel) has been used as a source of traditional medicine where herbal preparations have served as a laxative, wound dressing, aphrodisiac, demulcent, laxative, energizer, anti-infective,

anti-inflammatory, anticancer and antidiabetic agents [14]. The aqueous extracts of leafy stems of the plant have been used to treat prostatitis [20]. Using the MTT cell viability assay, researchers have also reported that purified protein from the tuberous root inhibits human colorectal cancer SW480 cell proliferation, migration, and invasion in a dose- and time-dependent manner [21]. Response surface methodology optimization of the acid extraction of pectin from crop residues showed remarkable antiproliferation effects on the human colon cancer cell line HT-29 and the human breast cancer cell line Bcap-37 by 46.64% and 42.64%, respectively, at 1.00 mg/mL separately, indicating that pectin could potentially act as a natural supplement in functional foods [22]. Oluyori et al. [23] fractionated an alcoholic extract of sweetpotato peel and discovered that the IB-F002C subfraction of the n-hexane fraction was the most active with IC_{50} values 24.75, 47.91, 52.37, 34.17, 46.07, and 25.89 µg/ml against breast, colon-1, colon-2, ovary, lung, and head/neck cancer cell lines, respectively. The major bioactive substances in the leaves of the root vegetable crop are flavonoids (hyperoside, kaempferol-3-O-glucoside, luteolin-7-O-glucoside, and quercertin-3-O-hexoside), phenolic compounds (3,4,5-tri-O-caffeoylquinic acid, 4,5-di-O-caffeoylquinic acid, caffeic acid), anthocyanins (peonidin 3-caffeoyl-p-coumaryl sophoroside-5-glucoside) and peptides [24]. Other phytochemical constituents, such as anthraquinones, alkaloids, oxalates, and steroids, have been found in the leaves at concentrations of 345.7, 328.4, 1.66 and 0.375 mg/100 g dry weight, respectively and smaller amounts of phytic acid, cyanide, saponins and tannins [25]. These bioactive substances exert anticancer effects alone or in combination with other compounds through the regulation of biochemical processes and signaling pathways, inhibition of enzymes necessary for cancer development, angiogenesis, microtubule assembly and induction of apoptosis [26].

The biological effect of compounds such as β-carotene, phenolic acids, and anthocyanins in sweetpotato include a unique variety of flesh (orange, purple, yellow, white) and skin (orange, purple, red, white, yellow, brown, pink) colors [27]. The color of this root vegetable crop may also be crucial to its health benefits. Teow [27] measured the antioxidant activities of 19 sweet potato genotypes with distinctive flesh colors (white, cream, yellow, orange, and purple) and found that the total antioxidant activity was greatest in the purple-fleshed sweetpotato cultivar. The author measured total anthocyanins using the pH-differential method, total phenolics with the Folin–Ciocalteau method, β-carotene by HPLC and antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH), oxygen radical absorbance capacity (ORAC), and 2,2′-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) [27]. Additionally, Ji et al. [28] with similar standard phytochemical content analysis methods reported that the dietary fiber content, total phenolic content, and total antioxidant capacity of purple-fleshed sweetpotato roots were significantly greater than those of sweetpotato roots of other flesh colors. The purple-colored tuberous root has been found to produce more stable anthocyanins than strawberries, red cabbage, and other plants [29]. These anthocyanins from purple root crops extracted by spray-drying and microwave-baking methods prior to invitro and in vivo assessment, were shown to have free radical scavenging, anti-aging, antihyperglycemic, antitumor, antimutagenic, and anticancer activities amongst others [30, 31]. In a study by Sugata et al. [32], purple-fleshed sweet potato ethanolic extracts were found to inhibit the growth of MCF-7 (breast cancer) and SNU-1 (gastric cancer) cancer cell lines using the 3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide (MTT) assay. Currently, nutritionists in the United States are researching the anticancer potential of anthocyanins in purple sweetpotato [33]. The orange color of sweetpotato, on the other hand, has been linked to higher levels of beta carotene that can protect the DNA in the cell's nucleus from substances that cause cancer outside the nuclear membrane [32].

The human body constantly reacts with oxygen as it breathes, and its cells produce energy [32]. Due to this activity, highly reactive molecules, known as free radicals, are generated within our cells, and oxidative stress occurs which may lead to inflammation and even cancer [34]. According to several epidemiologic reports, lung cancer is a significant cause of cancer related deaths in the United States [35] and worldwide; 80 to 90% of lung cancers occur because of tobacco use [36, 37], and exposure to secondhand tobacco with risk increasing with a decrease in age of exposure [38]. Other causes of lung cancer include pollution, family history, occupational exposure (e.g., arsenic, asbestos, nickel, and chromium), ionizing radiation (radon), age, acquired lung disease, and male sex [39]. A total of 1.82 and 1.59 million cases of lung cancer and deaths, respectively, were recorded worldwide in 2012. The countries with high human development indices had the highest incidences [40]. There were 210,828 recorded cases and 157,423 mortalities in 2012 in the United States [41]. Lung cancer occurs because of genetic damage to normal lung cells, leading to uncontrolled cell division [42]. Lung cancer can spread rapidly to neighboring tissues and cells or even to more distant parts of the body, eventually leading to the death of the individual if left without treatment [42]. Lung cancer is also referred to as bronchogenic cancer, as most of these cancers originate from the bronchi airways of the lungs [42]. The two main types of lung cancer cells

are small cell lung cancer (cancerous epithelial cells are abnormally small, also called oat cell carcinoma or small cell undifferentiated carcinoma) and non-small cell lung cancer (adenocarcinoma, squamous cell carcinoma, and large cell carcinoma) [42]. Although the type of lung cancer differs from country to country, adenocarcinoma occurs more commonly than squamous cell carcinoma, especially among women [40]. The 5-year survival period was relatively variable, ranging from 2% in Libya to 30% in Japan, with notable differences among countries [40]. The chances of improving lung cancer survival are a significant issue in modern medicine because, on average, the chances of 5-year survival are 15%, with only 7% of patients alive up to 10 years after being diagnosed [43]. Most of the time, this is because patients experience symptoms in the later advanced stages of the disease, and by the time of discovery, it has already spread to distant areas, coupled with the rate of cure being low [43]. Breast cancer on the other hand is the second leading cause of cancer among women after lung cancer and the risk factors include being a woman and getting older [44]. Each year approximately 240,000 women and 2100 men are diagnosed; 42,000 women and 500 men die from this cancer of the breast [44]. It can begin in the ducts or lobules of the breast where it spreads to other tissues and subsequently to other parts of the body [44]. Surgery is the most common treatment method for cancer, but other options include adjuvant chemotherapy, combined chemoradiotherapy, radiotherapy [45] hormonal therapy, biological therapy, and targeted therapy [44]. Herbs, medicinal plants, and other natural plant products such as sweetpotato in the diet or as a drug can serve as a natural alternative because they may contain some bioactive compounds or properties that can prevent the risk or spread of developing cancer.

Among the known health benefits of phytochemicals in orange and purple sweet potatoes, their free radical scavenging and antioxidant capacities are the most widely known [8]. However, their anti-inflammatory and anticancer activities have yet to be well studied [8]. Few studies have been done on the anticancer potential of sweetpotato leaves compared to the roots to determine how different sweetpotato cultivars would affect the viability of cancer cells. Although the entire sweetpotato plant (tuberous roots, leafy greens, and stem) have been used as a source of traditional medicine for centuries [14, 20]. No study has been done using sweetpotato stems alone or in combination with leaves. As an increasing number of people become aware of the nutritional health properties of sweet potato leafy vegetables, the relationship between diet and health is becoming increasingly understood. The overall objective of this paper was to determine the medicinal properties of two cultivars of sweet potato grown in the United States. In the present study, we obtained the tops (leaves and stems) of both sweetpotato cultivars from the Tennessee State University Certified Organic Farm in Nashville, TN. We hypothesized that the leaves and stems of the cultivars may have anticancer effects due to the presence of bioactive compounds, such as anthocyanins and polyphenols. To prove this, we included two controls: DMSO- (a positive control with no effect on the cell lines) and a drug called tamoxifen (a negative control with a detrimental effect) with sweet potato extracts. The chemo preventive effects of the leaf and leaf/stem extracts of All Purple (purple-fleshed) and Carolina Ruby (orange-fleshed) sweet potato strains were tested on the human lung (A549) and breast (BT549) cancer cell lines.

Methods

Collection of sweetpotato plant material and extract preparation: Sweetpotato plant materials (provided in Table 1) were collected from the organic farm of Tennessee State University and prepared for methanolic extraction. Two types of cancer cell lines (BT549 and A549) were used to evaluate the antiproliferative activity of the crude extracts; from the human breast and non-small cell lung adenocarcinoma (glandular) epithelial cells. The Alamar blue cytotoxicity assay was used to detect cell viability. Quantitative analysis was subsequently conducted after which the treatment differences, dose–response curves and half-maximal effective concentrations were determined.

Extraction procedure

Permission to collect sweetpotato was obtained from the Tennessee State University Certified Organic Farm. The plant specimens (the list of plants studied is highlighted in Table 1) were identified by Clifton Slade of Slade Farms, Dr. Dilip Nandwani, and Dr. Sochinwechi Nwosisi. The collected specimens were deposited at the Department of Biological Science, Tennessee State University, Nashville, Tennessee, USA. They were washed with distilled water, ground using a mortar [17] and diluted with methanol. Soxhlet was used to extract the plant parts (leaf/bark) for approximately 8 h. Methanol (1:40) containing leaf or bark was pre-weighed in a round bottom flask. Freshly weighed leaves (1.93 g) from the Carolina Ruby cultivar was extracted with 8.6 ml of solvent, while 0.25 g fresh weight leaves/stem was extracted with 2 ml of solvent from the All Purple sweetpotato cultivar. The authors removed the supernatant with a 0.45 µm syringe filter and rotary evaporator used for solvent evaporation. The concentrated extract was collected and stored at −20 °C until further use.

Table 1 Characteristics of the roots and vines of sweetpotato varieties used in this study

Plant Characteristics	All Purple Sweetpotato variety	Carolina Ruby variety
Primary skin color	Dark purple	Red
Secondary skin color	Light purple	Purple Red
Primary cooked color	Dark purple	Dark orange
Secondary color cooked	Intermediate orange	Intermediate orange
Vine pigmentation	Green with many purple spots	Green with few purple spots
Shape of central leaf lobe	Semi-elliptic	Absent
Abaxial leaf vein pigmentation	Green	Main rib partially purple
Mature leaf color	Green	Slightly purple
Immature leaf color	Green	Mostly purple
Petiole pigmentation	Green with purple at both ends	Green with purple at both ends
Leaf lobes number	3	1
Predominant storage root flesh color	Dark purple	Dark orange
Secondary storage root flesh color	Light purple	White
Distribution of secondary flesh color	Ring and other areas of the flesh	Covering most of flesh

Cell lines and chemicals

Alamar Blue was obtained from BioSource (Camarillo, CA, USA, cat. # DAL1025); and resazurin sodium salt (cat. #R7017) and L-ascorbic acid (cat. #A5960) were obtained from Sigma (St. Louis, MO, USA). DMEM and RPMI-1640 media supplemented with two mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin; fetal bovine serum, a solution of trypsin–EDTA and TPP 96-well tissue culture microplates purchased from CultiLab (Campinas, S.P., Brazil). The phosphate-buffered saline used in the preparation of resazurin solutions was obtained from Sigma. Dimethyl sulfoxide (DMSO) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Cytotoxicity and antiproliferative activity were determined using human cell-based screening systems; cell lines representing lung (A540) cancer and breast (BT549) cancer sources were obtained from the American Type Culture Collection (Rockville, MD).

Cell culture

The A549 (lung cancer) and BT549 (breast cancer) cell lines were obtained from the American Type Culture Collection (ATCC) (Rockville, MD). The cells were maintained in RPMI-1640 supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, and 1% penicillin–streptomycin. The human breast adenocarcinoma BT-549 (ATCC No. HTB-122) cell line was grown in RPMI-1640 and supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine and 1% penicillin–streptomycin. A549 (ATCC No. CCL-185) lung cancer cell lines were cultivated into DMEM medium, a low glucose variant (Gibco), encompassing 2 mM L-alanyl-L-glutamine, non-essential amino acids, penicillin–streptomycin, 5% fetal calf serum (Atlanta Biologicals) and supplemented with 0.01 mg/mL insulin and 1 mM sodium pyruvate. The cells were grown in T-75 culture flasks, in a 5% CO2 humidified incubator with 95% air at 37˚C and passaged biweekly after reaching 80% confluency in 1:2/1:4 ratios for no more than 40 passages.

Sample preparation

Stock solutions of methanolic extracts of All Purple sweetpotato leaves and Carolina Ruby leaves/stems were prepared in 100% dimethyl sulfoxide (DMSO) at 10 mg/ml and heated or sonicated if necessary to dissolve the sample before stock storage at –20 °C.

Cell viability assay: Alamar blue assay for 50% inhibition concentrations of IB extract for cancer cell growth

Cell viability assays help screen test compounds to determine whether they affect cell proliferation or cytotoxicity. After concluding the experiment, the aim was to determine how many cells were still alive. Alamar Blue, which is the cell viability reagent used in this study, behaves as an indicator of healthy cells by using the ability of living cells (animal, plants, bacteria, fungi) to be reduced in measuring the proliferation of cell lines in-vitro and enabling us to determine the relative cytotoxic potential of agents within different chemical classes. Living cells maintain a reducing environment within the cellular cytosol. Resazurin (Fig. 1), the primary bioactive compound in Alamar blue reagent, is a blue, nonfluorescent, nontoxic, cell-permeable compound that is reduced to resorufin (a combination that is pink/red in color and highly fluorescent) when it enters cells.

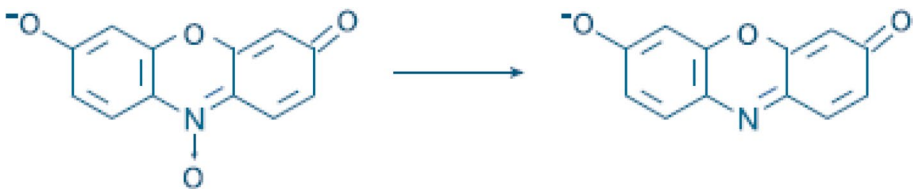

Fig. 1 The structure of the blue resazurin substrate was reduced to the pink resorufin product substrate in viable cells [46]

The incubation period should be optimized and kept short enough to avoid reagent toxicity but long enough to provide adequate sensitivity [47]. The main advantage of the Alamar blue assay is that it is not expensive, is more sensitive than the tetrazolium assays, makes use of a homogenous format, and can be combined with other methods like caspase activity measurement to understand better the mechanism that leads to cytotoxicity [47]. Its limitation is that the substrate needs to be incubated with viable cells at 37 °C for an optimum period, long enough to produce sensitivity to provide a signal and short enough to prevent the reagent from being toxic to the cells [47].

The 50% inhibition concentration of the extract for each cancer cell line was determined using the Alamar Blue Assay. The compound was further diluted to the appropriate concentration using the complete medium for the assays. All Purple sweetpotato leaf extracts were diluted to concentrations of 0.00005, 0.00009, 0.00019, 0.00039, 0.00078, 0.00156, and 0.00313 µg/µl for cell viability assays using Alamar blue. The concentrations of the methanolic extracts of the sweetpotato leaves/stem of Carolina Ruby sweetpotato dilution were.00009, 0.00018, 0.00035, 0.0007, 0.0014, 0.0028, and 0.0056 µg/µl. Tamoxifen at a concentration of 5 mM was used as the positive control. For the blank, 20 µl of Alamar Blue dissolved in 180 µL of DMEM was added to a well in a 96 well plate. Subsequent wells containing 60 µL of Alamar Blue were diluted in 465 µL of complete DMSO medium and incubated in triplicate with 15 µL of each of the test compounds: DMSO (negative control), the different concentrations of the sweetpotato methanolic extracts and tamoxifen. After gentle mixing, 15 µl of culture medium supplemented with 4×10^4 cells were added to each well. DMSO was added to each well to solubilize the formed formazan crystals [37]. There were three replications for each experiment except for the blank. The investigation was re-conducted with 525 µL of complete DMEM and no cells. Finally, the 96-well microculture plates were incubated in a CO2 incubator (37°C) for 24 h. After gentle shaking for 10 min, the absorbance was measured at 570 nm on a fluorescence spectrophotometer. The absorbance of treated cells was compared with that of control cells.

Statistical analysis

The authors used the mean ± standard error (Microsoft Excel) to present the results. Statistical differences between correlated samples were evaluated using paired Student t-tests (SAS software). The alamar blue assay results revealed the expression of a percentage of viable cells compared to that of the untreated controls. Concentration–response curves were generated by nonlinear regression curve fitting using sigmoidal dose–response stimulation (with variable slope) (GraphPad Prism 7.03 software). The EC_{50} is the concentration of the cytotoxic agent that led to a decrease of 50% of the signal.

Results and discussion

In summary, the proliferative potential of the different extract concentrations is presented as EC_{50} values and regression coefficients (R^2 values) (Fig. 2). The results obtained from the comparisons showed that Alamar Blue produced lower EC_{50} values for all purple leaf extracts in their interaction with the breast cancer cell line (BT549). However, the reverse was the case when observing the leaf and stem extracts of the Carolina Ruby cultivar. The authors observed a lower EC_{50} value in the Carolina Ruby cultivar's lung cancer cell line (A549). Similar to research by Kang et al. [17] where the antiproliferative potential of sweetpotato methanolic leaf extract varied for different cell lines, our study suggested that the effects of natural products effects vary with cancer cell line and that there seems to be a specific connection between natural products and cancer inhibition.

Compared with that of the A549 cell line, the All Purple sweetpotato leaf extract in the BT549 cancer cell line showed the highest anticancer activity at 0.0031 µg/

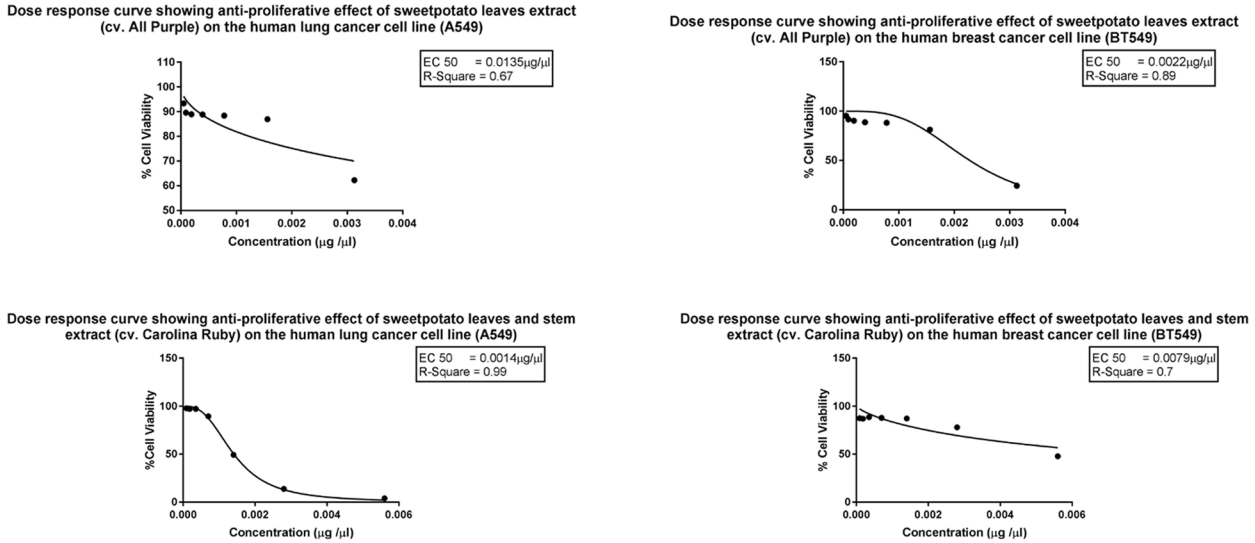

Fig. 2 Concentration–response curves of (**A**) Sweetpotato leaf extracts (cv. All Purple) in A549 cells (**B**) Sweetpotato leaf extracts (cv. All Purple) in BT549 cells (**C**) Sweetpotato leaf extracts (cv. Carolina Ruby) in A549 cells, and (**D**) Sweetpotato leaf extracts (cv. Carolina Ruby) in BT549 cells after 24 h of incubation using the Alamar Blue assay. The data shown are the means of 3 independent experiments

µl (75.65% greater than that of the control). The high R^2 value (0.89) was observed for BT549, with a half-maximal effective concentration (EC_{50}) value of 0.002 µg/µl compared with that of A549 cells (0.013 µg/µL) which had an R^2 of 0.67. Extracts of sweet potato leaves and stems of the Carolina Ruby sweetpotato cultivar exhibited potent anticancer activity with EC_{50} values of 0.0014 and 0.007 µg/µl and R^2 values of 0.99 and 0.7 against the A549 and BT549 cell lines, respectively.

No significant inhibition was observed by the sweetpotato leaf extracts of the All-Purple cultivar at concentrations less than 0.0009 µg/µl in the A549 cell line or less than 0.00078 µg/µl in the BT549 cell line (Fig. 3). The viability of A549 cells treated with All Purple leaf extracts at 0.00019, 0.00039, 0.00078, 0.00156, and 0.00313 µg/µl for 24 h was 11.06, 11.16, 11.61, 13.06 and 37.76% respectively greater than that of the DMSO (negative control) treated cells. The survival of BT549 cells treated with 0.00156 and 0.00313 µg/µl extract for 24 h was 18.76% and 75.65% higher, respectively, than that of DMSO treated cells. Compared with those cells treated with DMSO (negative control), the percentages of living A549 and BT549 cells treated with five mM of tamoxifen (positive control) decreased by 96.63% and 92.42%, respectively. Treatment of different breast cancer cell lines with purple sweetpotato (PSP) leaves has been shown to have significant effects on cancer antiproliferation in a 2021 review article [48].

For the Carolina Ruby cultivar stem/leaf extracts, the repression of A549 cells occurred at concentrations above 0.0014 µg/µl. At a concentration of 5 mM, the inhibition caused by tamoxifen on the A549 and BT549 cell lines was 95.11% higher than that of untreated DMSO cells. The experimental procedure performed with 0.0014, 0.0028, and 0.0056 µg/µl of Carolina Ruby stem/leaf extracts indicated that at those concentrations, inhibition of cell growth was at 50.67%, 86.93% and 96.07% higher than that of the DMSO control cells, respectively. Comparable results have been reported in which broccoli leaf and stem methanolic extracts showed a high level of polyphenol, antioxidant and anticancer activity in the colon cancer cell line HT-29 and the lung cancer cell line NCI-H1299 cell lines according to the MTT assay [49].

A review summarizing the bioactive compounds in SPL and their health benefits documented the recognition of the vegetable root crop as an anticancer food source against various cell lines [25]. In this present study, we evaluated the effects of sweetpotato extracts (cv All Purple leaf and Carolina Ruby leaf/stem) to test their potential anticancer activity. We investigated the cell viability of human lung A549 and breast BT549 cancer cells. The results showed a dose-dependent inhibition of cellular growth. The results showed that after treatment with All Purple (leaf extracts) and Carolina Ruby (leaf/stem extracts) sweet potato for 24 h, the growth of the A549 and BT549 cell lines was not significantly affected by lower concentrations of the extracts and was inhibited by higher concentrations. There was no significant difference in toxicity between some treatment groups.

Our results showed that the cell growth inhibition rate reached 37.6% and 76.65% when A549 and BT549 were treated with 0.00313 µg/µl of All Purple sweetpotato leaf

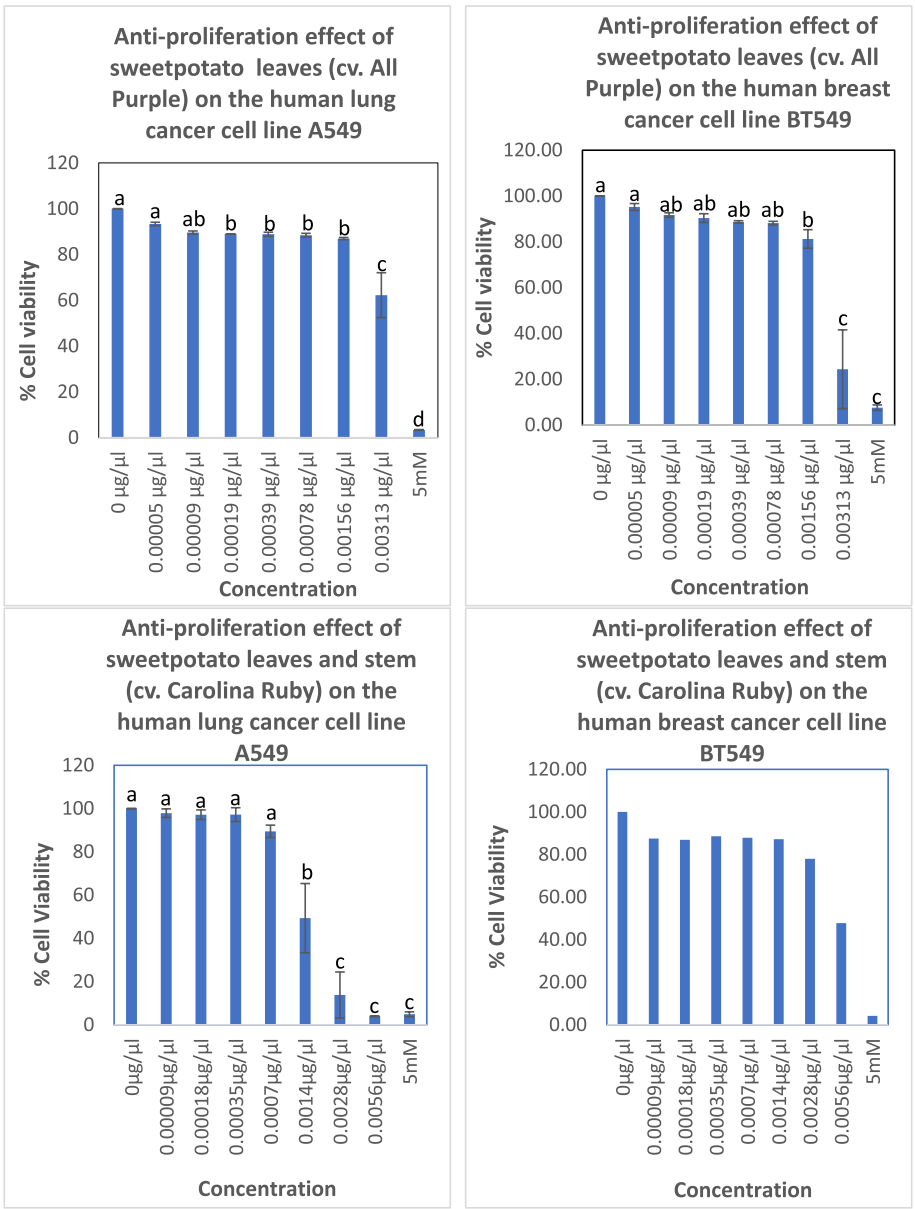

Fig. 3 Concentration–response curves of (**A**) Sweetpotato leaf extracts (cv. All Purple) in A549 cells (**B**) Sweetpotato leaf extracts (cv. All Purple) in BT549 cells (**C**) Sweetpotato leaf extracts (cv. Carolina Ruby) in A549 cells, and (**D**) Sweetpotato leaf extracts (cv. Carolina Ruby) in BT549 cells after 24 h of incubation using the Alamar Blue assay. The data are presented as the means ± SEMs of 3 independent experiments. Panels with the same letters are not significantly different at $P < 0.05$ (Paired t-test-LSD)

extracts for 24 h, which means that the sweet potato all purple leaf extracts had a greater effect on the breast cancer cell line BT549 than on the lung cancer cell line A549 at an equivalent concentration of 0.00313 µg/µl without significantly varying from the tamoxifen positive control cells. It is important to note however that no significant inhibition was observed by sweetpotato leaf extracts of the All-Purple cultivar at concentrations less than 0.0009 µg/µl in the A549 cell line or less than 0.00078 µg/µl in the BT549 cell line. Similarly, an MTT assay revealed that at a concentration of 0.0001 µg/µl, the sweet potato leaf extract inhibited proliferation in the African American breast cancer cell line, MDA-MB-468 [15]. Another closely related study revealed that methanol extracts of SPL inhibited the proliferation of all prostate cancer cell lines (PC-3, C4-2B, C4-2, DU145, and LNCaP) with IC_{50} values ranging from 145–315 µg/mL

due to modulations of the cell cycle, induction of apoptosis, and reductions in clonogenic survival [18].

The therapeutic potential of purple-fleshed sweet potato is ascribed primarily to its high anthocyanin content. Purple-flesh 33 sweetpotato (PSP) contains a significantly higher amount of anthocyanin than the ordinary, orange flesh sweetpotato (OSP) [50]. Anthocyanins or anthocyanin-rich extracts have been shown to inhibit cancer cell growth in various cancer cells [51]. In a study by Vishnu et al. (2019), the anthocyanins found in the root tuber and leaves showed potential in inhibiting the growth of MCF-7, HCT-116, and HeLa cancer cells, and their structure played a crucial role in this process. In experiments conducted in the lab, Hoechst 33,342 Live Cell Staining revealed that the anthocyanins from purple sweet potato root tuber and leaves did not exhibit any toxicity to cells at various concentrations (100–400 µg/mL) [52]. From a nutritional viewpoint, acylated anthocyanins possess elevated antioxidant and antimutagenicity activity [53]. Therefore, the acylated anthocyanins in the leaves may be more active at that concentration, or other phytochemicals, such as the phenolic acids therein, may interact synergistically [54].

Truong [55] compared the phenolic acid content in the roots, leaves, and peels of commercially important sweetpotato cultivars in the United States and detected the highest phenolic acid, 3, 5-di-O-caffeoylquinic acid and 4, 5-di-O-caffeoylquinic acid contents in the leaves. Studies have shown that polyphenolic compounds, caffeic acid, and di- and tricaffeoylquinic acids isolated from sweet potato leaves can suppress the growth of human cancer cells, stomach cancer (Kato III), colon cancer (DLD-1), and promyelocytic leukemia cells (HL-60) [13]. In recent years, research on the carcinogenic potential of flavonoids such as quercetin has revealed their potential as anticancer agents [4]. Research studies within and outside laboratory settings have shown the ability of quercetin to inhibit the viability of leukemic, colon, and ovarian carcinoma cells, especially human breast cancer cells [56, 57]. Ojong [58] identified and classified five common flavonoids in storage roots and leaves of 10 sweetpotato cultivars and, found that quercetin was present and much more concentrated in the leaves than in the storage roots. The results from previous studies suggest that these bioactive compounds induce apoptosis in cancer cells [6]. The bioactive compounds contained in the leaves of this vegetable root crop promote health by improving immune function, and reducing oxidative stress damage from free radicals, thereby decreasing the risk of cardiovascular disease, and suppressing cancer cell proliferation [6].

The cell growth inhibition rate of the Carolina Ruby stem and leaf extracts reached 96.07% when the A549 cells were treated with 0.0056 µg/µl extract, with an EC_{50} value of 0.0014ug/µl. This percentage was approximately 1% greater than the inhibitory rate of 95.11% observed in tamoxifen-treated cells. Interestingly, the inhibitory effect of 0.0056 µg/µl of the sweetpotato (cv Carolina Ruby stem/leaves) extract was superior to that of 5 mM tamoxifen at an effective concentration in the A549 lung cancer cell line. Our research findings suggest that leaf and stem extracts may induce apoptotic processes in cancerous cells and help suppress the growth of cancer cells. Our results agree with the antimutagenic, and anticancer properties of polyphenols contained in sweet potato leaves, which can prevent the mutation of normal cells into cancerous cells and suppress the growth of cancer cells, respectively [6]. Several anticancer mechanisms of anthocyanins may be involved, such as their potent antioxidant and anti-inflammatory properties, and ability to induce apoptosis by regulating the cell cycle in cancers [59]. Additionally, the anti-inflammatory effect of anthocyanins may play an essential role in cancer prevention [60]. Studies have shown that anthocyanin treatments may inhibit cell growth and induce apoptosis in cancer cells by interrupting the cell cycle at the G1 and G2/M phases [61]. However, the involved mechanisms still need to be determined, and the results differ depending on the tested anthocyanins from various sources [60]. Further rigorous studies are required to determine the potential mechanisms involved in the inhibition process and the extrinsic and intrinsic pathways through which this occurs.

To determine the EC_{50} values obtained with Alamar Blue, we tested for the cytotoxicity of the leaf/stem extracts on breast and lung cancer cell lines by cell necrosis, the first step in determining the safety of our compound. No or insignificant cell death/apoptosis means that our test compound (as observed in DMSO Fig. 3) either did not have harmful chemicals or did not have enough of them to cause acute effects [60]. On the other hand, a positive result could mean that our sample material has one or more substances that may not be safe. The risk of this substance can be observed and measured at the molecular, subcellular, or cellular level after examining several organizational levels, tissues, organs, or the entire organism [60]. For example, sweet potato is available in powder and capsule forms [62]. Dosage regimens vary, but most commercial manufacturers suggest the use of two capsules 30 min before meals, and up to 6 capsules daily [63]. Clinical studies of the efficacy of the nutraceutical caiapo, a sweet potato extract, used four tablets daily, with each capsule containing caiapo 168 to 336 mg [63].

The cytotoxicity of the alamar blue assay provides a fast and qualitative estimate of the potential danger of testing

sweetpotato leaf extracts classified as being active, and they can contribute to cutting research and development costs [64, 65]. As few toxicity data are available for the plant [63], these data will provide a framework for investigations leading to the development of new therapeutic agents. Not many other studies have studied the anti-cancer properties of the *Ipomoea batatas* leaf [13, 66]. Most focus on the anti-cancer qualities of the sweetpotato root. In the United States, there is limited consumption of sweetpotato leaves and stems. There have not been many studies assessing the health benefits of sweetpotato leaves and stems in preventing diseases such as cancer [13, 18, 52]. Despite being grown commercially and in farms, majorly in the Southeastern United States [6], the underutilized leaves and stems could offer sustenance for a growing population and possess health-promoting properties that have not been thoroughly researched. These properties arise from their bioactive compounds, majorly their abundant polyphenol content found in leafy green vegetables [52]. Thus, the results of this present study would encourage the consumption of sweetpotato in the diet of individuals in homes and communities as a preventive method and means to manage diseases.

Conclusions

Our findings showed that the All-purple methanolic leaf extracts and the Carolina Ruby cultivar methanolic leaf/stem of sweetpotato inhibited breast and lung cancer cell growth. Incorporating these leafy greens into our diet and the bioactive compounds isolated from the plant could serve as a protective strategy against cancer. These results suggest that the leaf and stem extracts are potential natural antioxidant and antitumor agents that can serve as drugs or functional food ingredients. Further studies are needed to investigate bioactive compounds, their synergistic interactions, their effects on gene expression and subsequent disease pathogenesis. The pharmacological action of sweet potato extract, cell morphology characteristics, cell cycle distribution, flow cytometry, clinical studies, and plausible medicinal applications of the crop (along with a safety evaluation) require additional research.

Acknowledgements
We are thankful to Tennessee State University Cooperative Extension for the financial support.

Authors' contributions
ELM and SN designed the experiments. SN performed cell assay experiments, analyzed the data and wrote the manuscript. ELM prepared crude methanolic extracts. ELM and DN supervised the study and reviewed the manuscript. DN obtained funding for the study.

Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request. Plant specimens were identified by Clifton Slade, Dr. Dilip Nandwani, and Dr. Sochinwechi. The plants were obtained from the Slade Farms, VA. The collected specimens were deposited at the Department of Biological Science, Tennessee State University, Nashville, Tennessee, USA.

Declarations

Ethics approval and consent to participate
Not Applicable.

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

Received: 1 December 2023 Accepted: 20 January 2025
Published online: 26 February 2025

References

1. Ayeleso TB, Ramachela K, Mukwevho E. A review of therapeutic potentials of sweet potato: Pharmacological activities and influence of the cultivar. Trop J Pharm Res. 2016;15(12):2751–61.
2. Van Jaarsveld PJ, Faber M, Tanumihardjo SA, Nestel P, Lombard CJ, Benade AJ. Beta-carotene-rich, orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response test. Am J Clin Nutr. 2005;81:1080–7.
3. Mohanraj R, Sivasankar S. Sweet potato (*Ipomoea batatas* [L.] Lam)-A valuable medicinal food: A review. J Med Food. 2014;17(7):733–41.
4. Ghasemzadeh A, Omidvar V, Jaafar HZ. Polyphenolic content and their antioxidant activity in leaf extract of sweet potato (*Ipomoea batatas*). Journal of Medicinal Plants Research. 2012;6(15):2971–6.
5. Mosha TC, Gaga HE. Nutritive value and effect of blanching on the trypsin and chymotrypsin inhibitor activities of selected leafy vegetables. Plant Foods Hum Nutr. 1999;54:271–83.
6. Johnson M, Pace RD. Sweet potato leaves: properties and synergistic interactions that promote health and prevent disease. Nutr Rev. 2010;68(10):604–15.
7. Islam, S: Medicinal and Nutritional Qualities of Sweetpotato Tops and Leaves. Cooperative extension program at University of Arkansas, Pine Bluff. N.D. https://www.uapb.edu/sites/www/Uploads/SAFHS/FSA-6135.pdf. Accessed 19 Apr 2024.
8. Sugata M, Lin CY, Shih YC. Anti-inflammatory and anticancer activities of Taiwanese, purple-fleshed sweet potatoes (*Ipomoea batatas* L. Lam) extracts. BioMed Res Int. 2015;2015:1.
9. Elgabry RM, Sedeek MS, Meselhy KM, Fawzy GA. A review on the potential health benefits of sweet potato: insights into its preclinical and clinical studies. Int J Food Sci Technol. 2023;58(6):2866–72.
10. Islam S. Sweetpotato (*Ipomoea batatas* L.) leaf: its potential effect on human health and nutrition. J Food Sci. 2006;71(2):R13–21.
11. Chandrika UG, Basnayake BM, Athukorala I, Colombagama PW, Goonetilleke A. Carotenoid content and in vitro bioaccessibility of lutein in some leafy vegetables popular in Sri Lanka. J Nutr Sci Vitaminol. 2010;56(3):203–7.
12. Abel C, Busia K. An exploratory ethnobotanical study of the practice of herbal medicine by the Akan peoples of Ghana. Alternative Med Rev. 2005;10(2):118.
13. Kurata R, Adachi M, Yamakawa O, Yoshimoto M. Growth suppression of human cancer cells by polyphenolics from sweetpotato (*Ipomoea batatas* L.) leaves. J Agricult Food Chem. 2007;55(1):185–90.
14. Parle M. Sweet potato as a super-food. International Journal of Research in Ayurveda and Pharmacy. 2015;6(4):557–62.
15. Johnson-Mayo I, Huang Z, Wang B, Pace R, Leggett-Robinson P, Troy R. Extracts from sweet potato greens inhibit tumor cell invasion and proliferation in African American breast cancer cells. Cancer Epidemiol Biomarkers Prevent. 2007;16(11_Supplement):B110.

16. Jin YR, Lee JH, Hsu HK, Lu JY, Chao SS, Kow-Tong Chen MD, Saou-Hsing Liou MD, Ger LP. Intake of vitamin A-rich foods and lung cancer risk in Taiwan: with special reference to garland chrysanthemum and sweet potato leaf consumption. Asia Pac J Clin Nutr. 2007;16(3):477.
17. Kang HG, Jeong SH, Cho JH. Antimutagenic and anticarcinogenic effect of methanol extracts of sweetpotato (*Ipomea batatas*) leaves. Toxicological Research. 2010;26:29–35.
18. Karna P, Gundala SR, Gupta MV, Shamsi SA, Pace RD, Yates C, Narayan S, Aneja R. Polyphenol-rich sweet potato greens extract inhibits proliferation and induces apoptosis in prostate cancer cells in vitro and in vivo. Carcinogenesis. 2011;32(12):1872–80.
19. Dhianawaty D, Panigoro R. Antioxidant activity and total weight of carotenoids in red sweet potato (*Ipomoea batatas* L.) tuber. Madurai, Int J Res Phytochemistr Pharmacol. 2013;3(1):70–3.
20. Emmanuel N. Ethno medicines used for treatment of prostatic disease in Foumban, Cameroon. Afr J Pharm Pharmacol. 2010N;4(11):793–805.
21. Li PG, Mu TH, Deng L. Anticancer effects of sweet potato protein on human colorectal cancer cells. World J Gastroenterol: WJG. 2013;19(21):3300.
22. Zhang YY, Mu TH, Zhang M. Optimisation of acid extraction of pectin from sweet potato residues by response surface methodology and its antiproliferation effect on cancer cells. Int J Food Sci Technol. 2013;48(4):778–85.
23. Oluyori AP, Shaw AK, Olatunji GA, Rastogi P, Meena S, Datta D, Arora A, Reddy S, Puli S. Sweet potato peels and cancer prevention. Nutr Cancer. 2016;68(8):1330–7.
24. Silva-Correa CR, Hilario-Vargas J, Villarreal-La Torre VE, Calderón-Peña AA, González-Siccha AD, Aspajo-Villalaz CL, et al. Potential Anticancer Activity of Bioactive Compounds from Ipomoea batatas. Pharmacognosy J. 2022;14(3):650–5.
25. Nguyen HC, Chen CC, Lin KH, Chao PY, Lin HH, Huang MY. Bioactive compounds, antioxidants, and health benefits of sweet potato leaves. Molecules. 2021;26(7):1820.
26. Ramakrishna W, Kumari A, Rahman N, Mandave P. Anticancer activities of plant secondary metabolites: Rice callus suspension culture as a new paradigm. Rice Sci. 2021;28(1):13–30.
27. Teow CC, Truong VD, McFeeters RF, Thompson RL, Pecota KV, Yencho GC. Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colours. Food Chem. 2007;103(3):829–38.
28. Ji H, Zhang H, Li H, Li Y. Analysis on the nutrition composition and antioxidant activity of different types of sweet potato cultivars. Food Nutr Sci. 2015;6(01):161.
29. Hwang YP, Choi JH, Yun HJ, Han EH, Kim HG, Kim JY, Park BH, Khanal T, Choi JM, Chung YC, Jeong HG. Anthocyanins from purple sweet potato attenuate dimethyl nitrosamine-induced liver injury in rats by inducing Nrf2-mediated antioxidant enzymes and reducing COX-2 and iNOS expression. Food Chem Toxicol. 2011;49(1):93–9.
30. Ahmed M, Akter MS, Eun JB. Impact of α-amylase and maltodextrin on physicochemical, functional and antioxidant capacity of spray-dried purple sweet potato flour. J Sci Food Agric. 2010;90(3):494–502.
31. Zhao JG, Yan QQ, Lu LZ, Zhang YQ. In vivo antioxidant, hypoglycemic, and anti-tumor activities of anthocyanin extracts from purple sweet potato. Nurs Res Pract. 2013;7(5):359.
32. The Cancer Cure Foundation. Cancer Fighting Foods/Spices. Newbury Park: Cancer Cure Foundation, 2010.
33. Anonymous. Facts and figures about the sweetpotato. International potato center. N.D. https://cipotato.org/site/worldfoodprize/pdf/FactsandFiguresSweetpotato.pdf. Accessed 19 Apr 2024.
34. Masaki H. Role of antioxidants in the skin: anti-aging effects. J Dermatol Sci. 2010;58(2):85–90.
35. Division of Cancer Prevention and Control. Lung cancer screening programmes: Policies and practices for cancer prevention. Centers for disease control and prevention Atlanta, GA. N.D. https://www.cdc.gov/cancer/ncccp/pdf/lungcancerscreeningprograms.pdf. Accessed 10 Dec 2015.
36. Jemal A, Thun MJ, Ries LA, Howe HL, Weir HK, Center MM, Ward E, Wu XC, Eheman C, Anderson R, Ajani UA. Annual report to the nation on the status of cancer, 1975–2005, featuring trends in lung cancer, tobacco use, and tobacco control. JNCI. 2008;100(23):1672–94.
37. Alberg AJ, Brock MV, Ford JG, Samet JM, Spivack SD. Epidemiology of lung cancer: Diagnosis and management of lung cancer: American College of Chest Physicians evidence-based clinical practice guidelines. Chest. 2013;143(5):e1S–29S.
38. Asomaning K, Miller DP, Liu G, Wain JC, Lynch TJ, Su L, Christiani DC. Secondhand smoke, age of exposure and lung cancer risk. Lung Cancer. 2008;61(1):13–20.
39. David P: The Management of Lung Cancer: ASTRO Refresher Course – La Jolla 2016. Western University, Canada, and Ontario institute for cancer research. 2016. https://www.astro.org/uploadedFiles/_MAIN_SITE/Meeting_and_Education/Events_(ASTRO)/2016/Sample_ASTRO_Meeting/Content_Pieces/ManagementofLungCancer-Palma.pdf. Accessed 10 Dec 2015.
40. Cheng TY, Cramb SM, Baade PD, Youlden DR, Nwogu C, Reid ME. The international epidemiology of lung cancer: latest trends, disparities, and tumor characteristics. J Thorac Oncol. 2016;11(10):1653–71.
41. U.S. Cancer Statistics Working Group. United States Cancer Statistics: 1999–2012 Incidence and Mortality Web-based Report. Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Cancer Institute, 2015. www.cdc.gov/uscs. Accessed 10 Dec 2015.
42. John TM. With every breath: a lung cancer guidebook. Lung Cancer Caring Ambassadors Program; 2005.484 p. http://www.lungcancerguidebook.org/lcguidebook_aug05/ch3_0605.pdf. Accessed 10 Dec 2015.
43. Crino L, Weder W, Van Meerbeeck J, Felip E. Early stage and locally advanced (non-metastatic) non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment, and follow-up. Ann Oncol. 2010;1(21):v103–15.
44. Centers for Disease Control and Prevention (CDC). Basic information about breast cancer. Division of Cancer Prevention and Control. US Department of Health and Human Services, 2023. https://www.cdc.gov/cancer/breast/basic_info/index.htm#:~:text=Each%20year%20in%20the%20United,cancer%20than%20all%20other%20women. Accessed 19 Apr 2024.
45. National Institute for health and care excellence (NICE). The diagnosis and treatment of lung cancer update. National Collaborating Centre for Cancer, Cardiff Wales, 2011. https://www.nice.org.uk/guidance/cg121/evidence/full-guideline-181636957. Accessed 10 Dec 2015.
46. Invitrogen. Alamar Blue Cell Viability Reagent. Molecular Probes Inc. MP 01025, 2008. https://assets.thermofisher.com/TFS-Assets/LSG/manuals/AlamarBluePIS.pdf. Accessed 19 Apr 2024.
47. Riss TL., Richard A, Moravec ALN, Sarah D, Hélène AB, Tracy JW, Lisa M. Cell viability assays. In: Sittampalam GS, Coussens NP, Brimacombe K, et al., editors. Assay Guidance Manual [Internet]. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences; 2004-. PMID: 23805433, 2016. https://www.ncbi.nlm.nih.gov/books/NBK144065/. Accessed 19 Apr 2024.
48. Budiman MR, Wiraswati HL, Rezano A. Purple sweet potato phytochemicals: potential chemo-preventive and anticancer activities. Open Access Macedonian J Med Sci. 2021;9(F):288–98.
49. Hwang JH, Lim SB. Antioxidant, and anticancer activities of broccoli by-products from different cultivars and maturity stages at harvest. Preventive nutrition and food science. 2015;20(1):8.
50. Xu J, Su X, Lim S, Griffin J, Carey E, Katz B, Tomich J, Smith JS, Wang W. Characterization, and stability of anthocyanins in purple-fleshed sweet potato P40. Food Chem. 2015;1(186):90–6.
51. Wang LS, Stoner GD. Anthocyanins, and their role in cancer prevention. Cancer Lett. 2008;269(2):281–90.
52. Vishnu VR, Raveendran SR, Archana M, Shirly RA, Janardanan S, Alummoottil NJ. Comparative study on the chemical structure and in vitro antiproliferative activity of anthocyanins in purple root tubers and leaves of sweet potato (*Ipomoea batatas*). J Agric Food Chem. 2019;67(9):2467–75.
53. Suda I, Oki T, Masuda M, Nishiba Y, Furuta S, Matsugano K, Sugita K, Terahara N. Direct absorption of acylated anthocyanin in purple-fleshed sweet potato into rats. J Agric Food Chem. 2002;50(6):1672–6.
54. Lim S, Xu J, Kim J, Chen TY, Su X, Standard J, Carey E, Griffin J, Herndon B, Katz B, Tomich J. Role of anthocyanin-enriched, purple-fleshed sweet potato p40 in colorectal cancer prevention. Mol Nutr Food Res. 2013;57(11):1908–17.
55. Truong VD, McFeeters RF, Thompson RT, Dean LL, Shofran BR. Phenolic acid content and composition in leaves and roots of common

commercial sweetpotato (*Ipomea batatas* L.) cultivars in the United States. J Food Sci. 2007;72(6):C343–9.
56. Davis W, Lamson MS, Matthew S, Brignall ND. Antioxidants and Cancer III: Quercetin. Alter Med Rev. 2000;5(3):196–204.
57. Gibellini L, Pinti M, Nasi M, De Biasi S, Roat E, Bertoncelli L, Cossarizza A. Interfering with ROS metabolism in cancer cells: the potential role of quercetin. Cancers. 2010;2(2):1288–311.
58. Ojong PB, Njiti V, Guo Z, Gao M, Besong S, Barnes SL. Variation of flavonoid content among sweetpotato accessions. J Am Soc Hortic Sci. 2008;133(6):819–24.
59. Soyoung L, Jaeyong K, Tzu-Yu C, Edward C, Jason G, Takeo I, John T, Betty H, Weiqun W, 2012. Anthocyanin- Enriched, Purple-Fleshed Sweet Potato for Potential Colon Cancer Prevention. School of Medicine, University of Missouri-Kansas City, Kansas City, MO, 64108, 2012. http://krex.k-state.edu/dspace/bitstream/handle/2097/13719/soyounglim2012.pdf..pdf?sequence=1. Accessed 10 Dec 2015.
60. Miret S, De Groene EM, Klaffke W. Comparison of in vitro assays of cellular toxicity in the human hepatic cell line HepG2. SLAS Discovery. 2006;11(2):184–93.
61. Yun JM, Afaq F, Khan N, Mukhtar H. Delphinidin, an anthocyanidin in pigmented fruits and vegetables, induces apoptosis and cell cycle arrest in human colon cancer HCT116 cells. Molecular Carcinogenesis: Published in cooperation with the University of Texas MD Anderson Cancer Center. 2009;48(3):260–70.
62. Anonymous. Centers for Disease Control and Prevention, National diabetes fact sheet: general information and national estimates on diabetes in the United States (2005), Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. 2005.
63. Dutta S. Sweet potatoes for diabetes mellitus: a systematic review. Pharmacophore. 2015;6(1):60–72.
64. Slater K. Cytotoxicity tests for high-throughput drug discovery. Curr Opin Biotechnol. 2001;12(1):70–4.
65. Ball SE, Scatina JA, Sisenwine SF, Fisher GL. The application of in vitro models of drug metabolism and toxicity in drug discovery and drug development. Drug Chem Toxicol. 1995;18(1):1–28.
66. Taira J, Uehara M, Tsuchida E, Ohmine W. Inhibition of the β-Catenin/Tcf Signaling by Caffeoylquinic Acids in Sweet Potato Leaf through down Regulation of the Tcf-4 Transcription. J Agric Food Chem. 2014;62(1):167–72.

Publisher's Note