

Efficacy of household spices on prevention of lung cancer cells.

By Samaara Bhardwaj

Abstract: Spices play an integral role in the culinary and cultural landscape alongside possessing medicinal properties. In the culinary world, spices are typically used as flavor enhancers that aim to make food more appealing. Spices are the foundation of culinary art and contribute to the diversity of flavors in global cuisines. In the medicinal landscape, spices have anti-oxidant qualities that reduce and neutralize the growth of free radicals in the body which causes cellular damage and increases the risk of cancer. In my experiment, I chose 8 compounds of spices that were readily consumed by households all over the world and tested their efficacy in killing the A549 lung cancer cell line. I did this with the hopes to find a pragmatic and affordable solution with no side effects to help people worldwide battle cancer.

Introduction

Spices taste good because they are good for us. Spices were used commonly during the Middle Ages as they used to trade them. Today spices are more important and are a prevalent part of many cuisines. Spices usually come from woody shrubs, vines, trees, aromatic lichens and roots, flowers seeds, and fruits of herbaceous plants. They have unique atoms and flavors that come from the compounds in them. Traditional cookbooks use spices more in meat-based recipes while today cookbooks have a combination of both vegetarian and nonvegetarian recipes.

Out of 188 species in the dictionary 152 species linked them to 848 unique disease specific MeSH. There is an exponential increase in the therapeutic benefits associated with spices after more and more research is being conducted on the efficacy of spices over a wide range of diseases, specially the ones that have matched in the database to a specific disease. The top disease categories which were negatively associated with spices were vascular diseases, skin diseases, hypersensitivity and respiratory hypersensitivity. There were positive relations between spices and most viral diseases. The beneficial aspects of spices are due to the presence of bioactive phytochemicals such as polyphenols.

Spices have antimicrobial properties and can inhibit the growth of some microorganisms before they can produce toxins which will reduce diseases like food poisoning. Typically spices inhibit 75 of the bacterial species. There is a speculation that cooking the spices may inhibit the antimicrobial effects and cooking may destroy the potency of phytochemicals. Spices also vary with climate. A greater proportion of bacteria is inhibited by receipt from hot climates than cool climates. Cooler climates contain less potent spices than cuisines from lower latitudes and elevations. There is a limit to how much of any one spice can be added before beneficial phytochemicals become phytotoxins. These spices and herbs have also been discovered to have medicinal properties. Most of the herbs and spices have a bio active component which is being investigated for potential disease prevention and treatment. These herbs are spiced and are considered safe by the FDA. Herbs and spices also potentially have antimicrobial and

antifungal activity. Some cuisines like Latin American and Chinese are becoming increasingly popular as people are becoming more health conscious as these cuisines have very low fat levels. Herbs and spices are also antioxidants which is important to maintain the cell membrane integrity, proteins, nucleic acids. Imbalance can cause cell death, low immunity and even cancer. Herbs and spices also reduce inflammation which reduces cancer as 15% of inflammations lead to cancer.

Natural products with anti-inflammatory and antioxidant properties in adequate doses can test the metabolic syndrome. Metabolic syndrome is when there is clustering of several cardiovascular and metabolic risk factors and increased blood pressure where obesity and insulin resistance are core parameters. This is common in adult populations throughout the world. Inflammation has been proposed as the critical process initiating the symptoms of the metabolic syndrome which is initiated by excessive caloric intake in a high carbohydrate and fat diet. *In Vitro* studies show that cardamom inhibits platelet aggregation when induced with some agents.

Cardamom reduces blood pressure in rats, probably by acting through cholinergic and calcium agents antagonist mechanisms. Cinnamon reduces fasting blood glucose concentrations, improves plasma lipid profiles in diabetic humans and reduces glucose and insulin responses in oral glucose tolerance testing. Coriander is usually used in gastrointestinal complaints. Cumin seeds widely used as ayurvedic medicine for treatment of dyspepsia, diphtheria and jaundice. Aqueous extract of the seeds lowered blood glucose and plasma and tissue lipid concentrations in allowance induced rats. Methi is recommended as treatment of rheumatism in old medicine. It reduces post pea dial blood glucose concentrations and improves insulin sensitivity. Garlic reduces blood pressure, blood lipid and glucose concentrations. Ginger helps stomach aches, diarrhea, nausea, asthma, respiratory disorders, toothache and many more problems. Haldi has anti inflammatory anti carcinogenic and anti microbial properties. Black mustard causes increasing thermogenesis and fat oxidation (2)

Spices and Cancer

Spices are used for flavoring and medicine for many years. This is because of their special properties like: antioxidant, anti-inflammatory and immunomodulatory effects which can prevent certain cancers. Cancer is one of the major causes of death in the world but surgery, chemotherapy and radiation helps mainly when the tumor is small. These therapies have many side effects which are not present in spices. Turmeric and cur cumin can help reduce nasopharyngeal, lung, hepatobiliary, breast, gastric, colorectal, prostate, uterus, hematopoietic tumors, and other cancers. Black cumin can help reduce the effects of similar cancers with additional pancreatic cancer. Ginger can help reduce the effects of similar cancers with prostate cancer additionally. Saffron, cinnamon, rosemary red chili pepper, capsaicin also help reduce effects of the same cancers. Spices are consumed in human diets in safe amounts and have no side effects so are a good method to reduce the effects and prevent some commonly fatal cancers.

Liver cancer can potentially be prevented by natural products like black currant plum pomegranate cruciferous vegetables french beans tomatoes and many others because their active components could affect the development of liver cancer cells. Diet plays an important role in determining overall cancer risks. Fruits have polyphenols which show high antioxidant properties which can reduce the risk of cancer. They have major bio active constituents which show anticancer potential according to studies. Additionally vegetables also help reduce risk of cancer in the digestive tract and liver cancer. Spices also contain compounds that have shown to induce liver cancer cell death. When natural products are used in combination with radiotherapy or chemotherapy it can improve the efficacy of the treatment. Some herbs like curcumin in the diet have reduced the risk of colon cancer.

Spices have been extensively studied for their medicinal properties, but there is limited research specific to the spices in the mediterranean diet. The spices that have chemotherapeutic and chemopreventive properties are ginger, pepper, rosemary, turmeric, black cumin and clove. Black pepper is a staple in the mediterranean diet and ginger and black cumin exhibit the highest anticancer activity targeting. Apoptosis induction is a common pathway that is activated by these spices. Spices and herbs have been used for a long time for flavor and for traditional medicine. The Mediterranean diet is abundant in phytochemicals and has many health benefits like reducing the risk of chronic diseases including cancer. The antioxidant and anti-inflammatory and immunomodulatory effects of the spices play a role in preventing and treating cancer through processes like apoptosis, cell cycle modulation and inflammation control. The plants for mediterranean regions are also used as food additives with a focus of enhancing taste, appearance, and preserving flavor while reducing microbial growth. Anticancer spices that are found in the Mediterranean diet are ginger, peppers, rosemary, turmeric, black cumin and clove.

There are certain transcription factors that regulate the expression of inflammatory gene products and are constitutively active in many cancers. Chronic inflammation results from conditions like pancreatitis, prostatitis and hepatitis. It leads to cell proliferation, survival, invasion, angiogenesis, metastasis and resistance to chemotherapy and radiation. Countries such as India exhibit lower cancer incidence compared to countries where spice consumption is lower like the US. Components of spices that have the potential to prevent these diseases are curcumin, gingerol, DAS, pinene, cineole.

Tulsi

Tulsi essential oil contains bioactive compounds that are potential alternatives for cancer treatment. Tulsi is used often in India for its religious and medicinal properties. Cancer can result from harmful mutations and it poses a global health challenge that has escalating incidence and death rates. Tulsi is undergoing an exploration for its potential in cancer treatment as it has more than 30 natural substances for various cancer types. The composition of Tulsi. Studies suggest that eugenol found abundantly in Tulsi leaves demonstrates anticancer effects by causing cell death, cell cycle arrest, inhibiting migration, metastasis and angiogenesis across various cancer types. It considers specific molecular targets and kills the cancer cell. Eugenol has antioxidant properties and it combats oxidative stress by scavenging free radicals. Tulsi also

has anticancer potential by inducing apoptosis and inhibits breast cancer spread, affecting carcinogen enzymes and demonstrating apoptotic activities.

Garlic

For what garlic showed bactericidal activity similar to antibiotics. This is done through the interference with the phospholipid bilayer of the membrane leading to higher permeability, loss of organelles and destruction of genetic material. *Helicobacter* species may facilitate the invasion and progression of cancer in the stomach, gallbladder and intestine. Compounds in garlic, pepper, rosemary, turmeric, and cinnamon appear to influence phase I and phase II enzymes. Garlic is recognized for its diverse pharmacological potentials including antibacterial, antiarthritic, antithrombotic, hypoglycemic and hypolipidemic effects with its anticancer properties being extensively researched. Breast cancer remains a significant contributor to global cancer deaths especially where incidence rates are rising rapidly. Garlic has bioactive compounds and they demonstrate preventative effects against breast cancer at many stages like initiation, promotion and progression. Their compounds affect cell signaling and the pathways the cancer cell takes which affects their growth and reduces it. This is in preclinical models and they need to fully understand the therapeutic potential of garlic for breast cancer management.

Cinnamon

Plants in the form of herbs and spices have been used in traditional medicinal practices for many many centuries. These spices contain anti microbial properties that are recently being used in the cosmetics industry as they are known to have a long shelf life and fight air borne pathogens. Cinnamon is one of the widely identified spices to have many health benefits because of its main compound called eugenol. It is not only used as a flavor additive it also improves oral health, treats acne and melisma, and many other physical problems. Studies are promoting the use of cinnamon applications in food and cosmetic products although in small doses. Further studies are being performed on its toxicity before its clinical use and on the antibacterial effect of the spice along with its ability to battle other infectious diseases.

Turmeric

Turmeric has been identified as a spice that decreases expression of RTKs. In vitro studies indicate herbs, spices and their bio active components can inhibit and sometimes induce pathways that regulate cell division for cell proliferation, detoxification in addition to the inflammatory immune response (1). Curcumin has many therapeutic benefits reducing multiple chronic diseases, inflammation, arthritis, metabolic syndrome, liver disease, obesity, neurodegenerative diseases and most importantly reducing the effect of many cancers. In today's day and age breast cancer is the prevalent and malignant tumor among the female population of the world. Curcumin interferes with the cell signaling pathway of cancer and acts on the proliferation of breast cancer cells. It shows the effects of anti-invasive activities to regulate the cancer cell and recently curcumin's ability to modulate the expression of some nucleotides in breast cancer cells has been found. It also has preventative and apoptotic effects

on lung cancer cells, hematological cancer cells, digestive cancer cells, pancreatic and hepatic cancer cells and other cancers like prostate cancer. Even though curcumin has many medicinal properties it has very limited bioavailability and low aqueous solubility, thus a mixed treatment of a cancer drug along with intake of curcumin will be an effective way to control certain cancers.

Animal studies documented the beneficial influence of turmeric, red pepper, and garlic on lipid metabolism especially hypercholesterolemic effect of the three spices and anti-lithogenic effect of curcumin and capsaicin. These spices have anti-diabetic effects, anti-oxidant properties, anti-inflammatory influence, digestive stimulant, anti-mutagenicity, anti-lithogenic properties, influence on platelet aggregation. They generally reduce LDL oxidation. Spices have traditional and medicinal use which have become clear by extensive animal studies in the past three decades. There are many physiological benefits attached to the consumption of spices but they don't contribute significantly to the nutrient makeup of the food because they are intaken in very small quantities.

Green Tea Catechins

Green tea catechins have antioxidant properties and can neutralize reactive oxygen species. Recently green tea catechins serve as the basis of anti-cancer drug synthesis. Green tea is famously drunk in China, Taiwan, and Japan. Green tea also has a high protein content, trace element content, and vitamin content (matcha is very high in vitamin A). It has many health benefits due to the reason that the manufacturing of green tea doesn't involve fermentation which does not affect the enzymes present in the tea. Green tea has antioxidant properties and antitumor properties along with chemopreventive properties. However, it cannot be an alternative for the treatment but it can enhance the effect and trigger cell death without killing body cells. Catechins are in the most stable environment which depends on pH. Catechins can detect copper which is commonly found in cancer cells which helps them detect the cancer cells and inhibit their growth. However, According to research, drinking five to nine cups of green tea in a day can increase the risk of bladder cancer thus the regulation of the intake of green tea catechins should be kept in mind.

Piperine

Cancer is usually due to the uncontrollable growth of cancer cells in the body. There are different stages of cancer to make sure the stages of cancers don't accelerate; there are treatments like chemoprevention techniques. Piperine can also modify the activities of enzymes and transcription to help reduce cancer in the body. Cancer develops very slowly and it goes through a transformation, it is broken down into three stages and it is necessary to prevent it from moving from stage to stage which piperine can help. Piperine induced the apoptosis of cancer cells, cell proliferation, cell cycle arrest, and modulations and detoxification. Piperine also alters the redox homeostasis in a cell and tissue-specific way to retard cancer development. Piperine influences signaling and induces apoptosis in UV-irradiated skin cells; it has chemopreventive potential. Piperine also inhibits glycoprotein activity as it enhances the apoptosis in cancer cells and also enhances the radiosensitization of otherwise resistant tumors and it doesn't affect the beneficial chemicals of fruits and vegetables.

Ashwagandha

Ashwagandha is widely known in the Ayurvedic group and has been used for many many years for medical illnesses. It is commonly known for stress management, energy elevation improving cognitive health and lowering inflammation blood sugar levels, and many other benefits. After being tested in many placebo tests it has been declared that ashwagandha is safe and tolerable with close to no side effects after intake. Studies have also shown WS to inhibit the growth of new cancer cells and programmed death of cancer cells. Additionally, several cancer types have shown an 80% inhibition while using WS in combination with chemotherapy. Specifically breast cancer, colorectal cancer, lung cancer cells, ovarian cancer, and other cancers like melanoma leukemia cervical cancer ashwagandha leads to growth arrest and apoptosis. At specific doses, WS extract has shown effectiveness and anti-cancer efficacy. It can be used in two ways to help reduce the risk of cancer. First it is in combination with radio and chemotherapy or secondly used as an adjunct therapy as it is very safe.

Cryptocarya concinna

CPC's efficacy on certain diseases was tested: oral cancer in particular. It was effective against two types of oral cancer. Oral cancer is the sixth most common cancer in the world and it's so easy to detect clinically. To treat this some anti oral therapies are also required. In the MTS assay the cell's viability of one of the oral cancer cell decreased. The other oral cancer cell remained similar to the control group. CPC can inhibit cell proliferation and apoptosis of oral cancer cells but is less harmful to normal oral cells.

Ginger

Ginger is a widely consumed dietary supplement and it has anticancer potential and its effectiveness varies based on geographical differences in bioactive compound levels. Study evaluated 22 ginger samples through efficacy based fingerprinting using different techniques on triple negative breast cancer cells. The results showed specific analytes correlating strongly with anticancer efficacy. This is a more streamlined method to identify potent anticancer compounds from ginger's chemical profile which are needed for these therapeutic benefits. The study shows the challenges of controlling the quality of natural medicines like ginger due to environmental factors that influence their compositions. By introducing an innovative approach the research establishes a relationship between the chemical fingerprint and the anti-proliferative effectiveness of ginger 22 extracts. This shows how the composition can vary and that a holistic control method is necessary to make the correlation.

Doxorubicin

Doxorubicin is recognised for its therapeutic potential and is widely used to treat various cancers. Although it has toxic effects like cardiotoxicity which are being studied. Despite the side effects, DOX remains a vital part of cancer therapy which is used in new formulations and drugs. Dox has many modes of action which include DNA damage, reactive oxygen species production, apoptosis. DOX mechanisms include DNA intercalation formation of DOX DNA adducts activating the DNA damage response pathway and topoisomerase trapping. DOX has a multifaceted action to cause DNA damage of the cancer cell to cause cell apoptosis. Researchers are developing special delivery systems like liposomes and nanogels to enhance

therapeutic benefits while minimizing toxicity. Understanding the interaction with the tumor microenvironment is crucial and coming DOX with immune checkpoint inhibitors needs to be explored for improved cancer treatment.

Materials and Methods

1. Extraction method

8 test compounds (Wheatgrass, Ginger, Garlic, Tulsi, Green tea, Ashwagandha, 95% extract of Piperine and Turmeric) were obtained from the formulation department of Dabur Research Foundation, Sahibabad, Ghaziabad, U.P. 201010.

100 mg of the test compounds was weighed and dissolved in DMSO. After that, it was vortexed thoroughly followed by centrifugation. The clear supernatant was then used for further experimentation.

2. Cell culture

Human Lung carcinoma epithelial cell line, A549 cell line was obtained from the National Centre for Cell Sciences (NCCS, Pune). The cell line was cultured in DMEM (Himedia), supplemented with 10% fetal bovine serum (Sigma-Aldrich), 25U/ml penicillin, and 25 mg/ml streptomycin (Himedia) at 37°C in a water-saturated atmosphere of 95% air and 5% CO₂.

3. MTT assay

The effect of DMSO extracts of 8 test compounds (Wheatgrass, Ginger, Garlic, Tulsi, Green tea, Ashwagandha, 95% extract of Piperine and Turmeric) on A549 human lung carcinoma epithelial cell line was observed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. MTT reagent was purchased from Sigma (Darmstadt, Germany). Cells were counted on a hemocytometer and 10,000 cells/well were plated in 96 well plate in 180µl of complete media (containing 10% of FBS).

4. Cells were treated with different concentrations (0.01, 0.1, 1, 10, 50, 100 µg/ml) of Wheatgrass, Ginger, Garlic, Tulsi, Green tea & Ashwagandha extracts and at concentrations (0.001, 0.01, 0.1, 1, 10 and 50 µg/ml) of 95% extract of Piperine and Turmeric for 72 hr. After the stipulated time, MTT solution (5mg/ml) was added to each well and incubated for 3hr. The purple-colored precipitate of formazan was dissolved in 150 µl of DMSO (Sigma-Aldrich) by proper mixing. The color absorbance of each well was recorded at 570 nm in a Biotek microplate reader with a reference serving as blank. Then, IC₅₀ values of DMSO extracts of 8 test compounds were calculated.

5. Morphological study under phase contrast

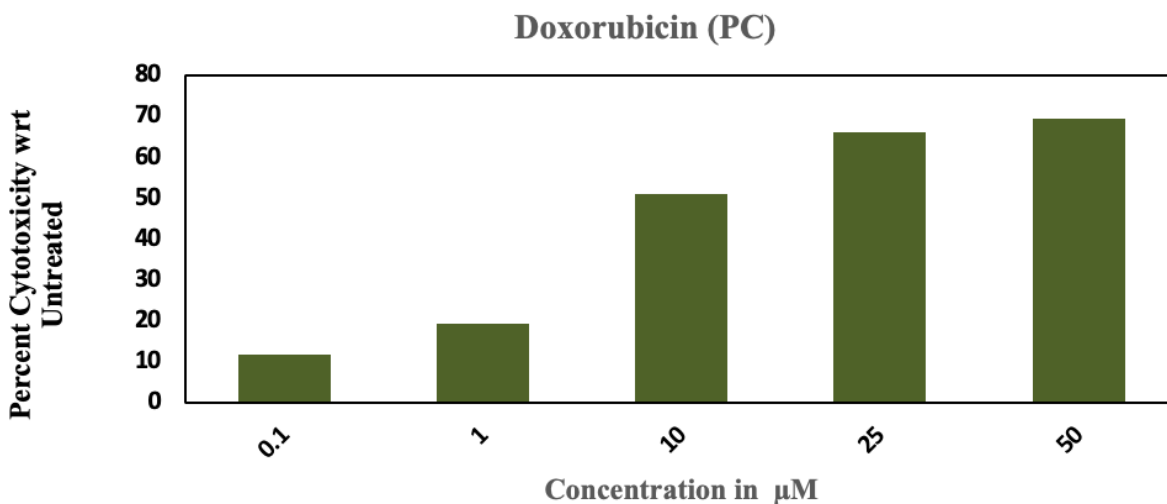
Phase contrast microscopy – A549 cells were treated with different IC₅₀ concentrations of DMSO extracts of 8 test compounds (Wheatgrass, Ginger, Garlic, Tulsi, Green tea, Ashwagandha, 95% extract of Piperine and Turmeric) for 72 hr.

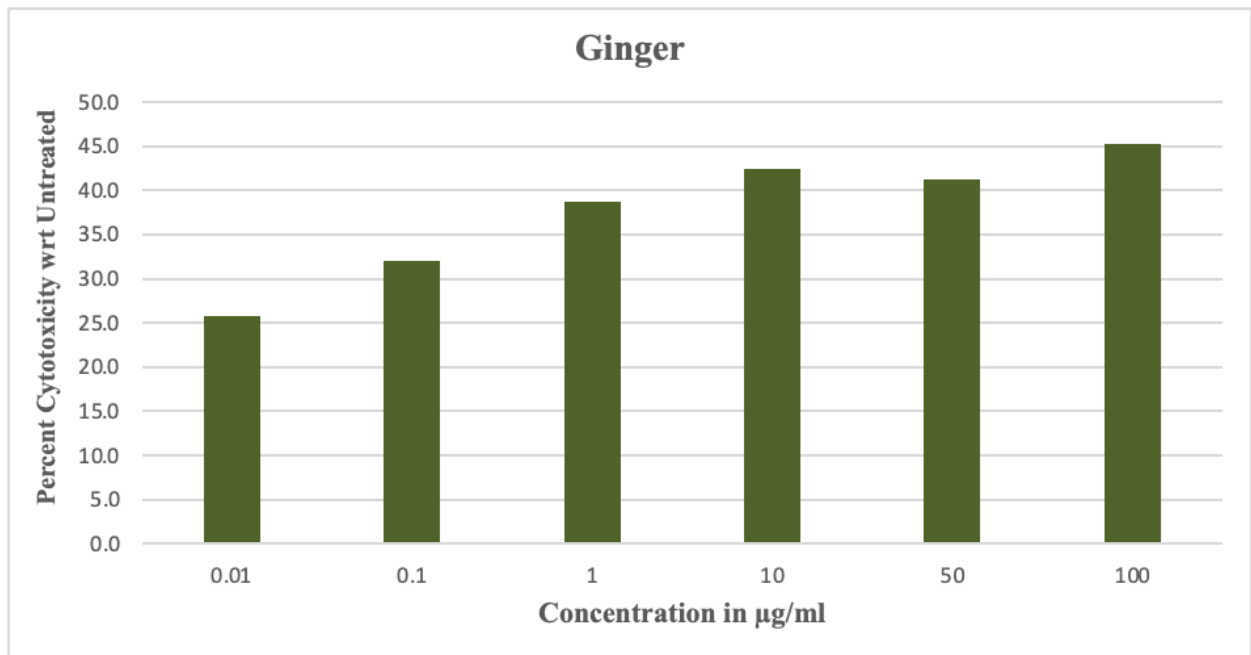
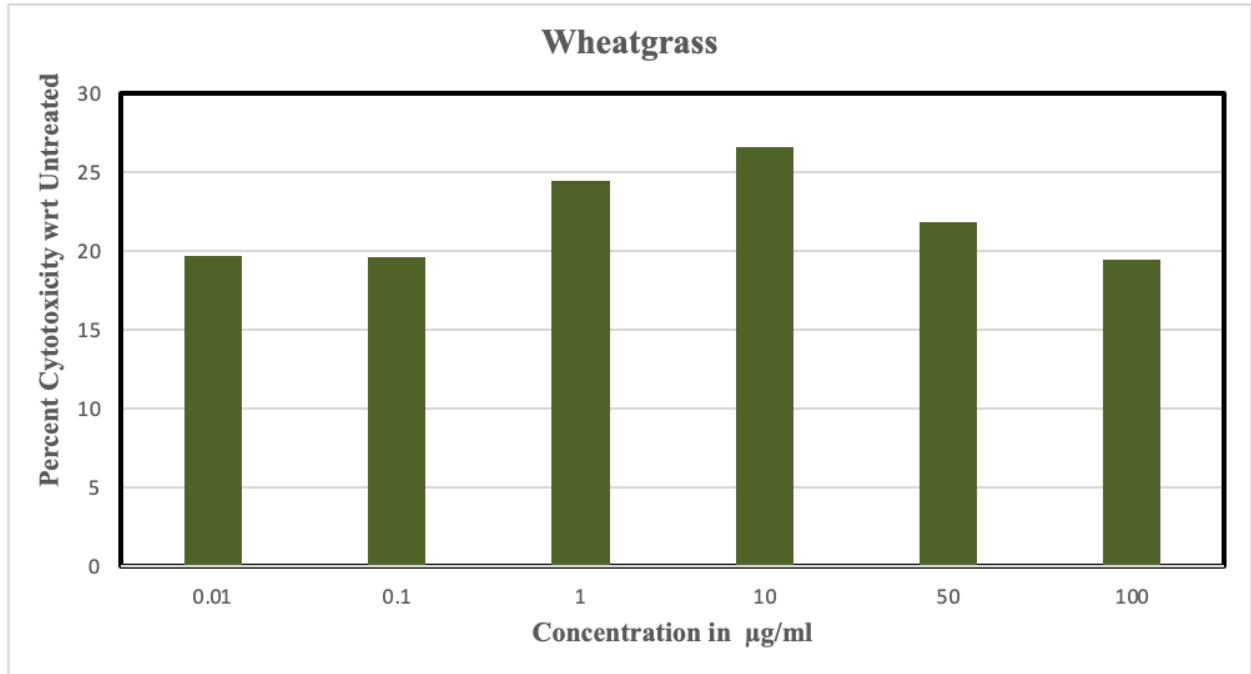
6.Following incubation, the cells were observed under phase contrast microscope at 20x Magnification. Pictures were acquired with the help of ToupView software.

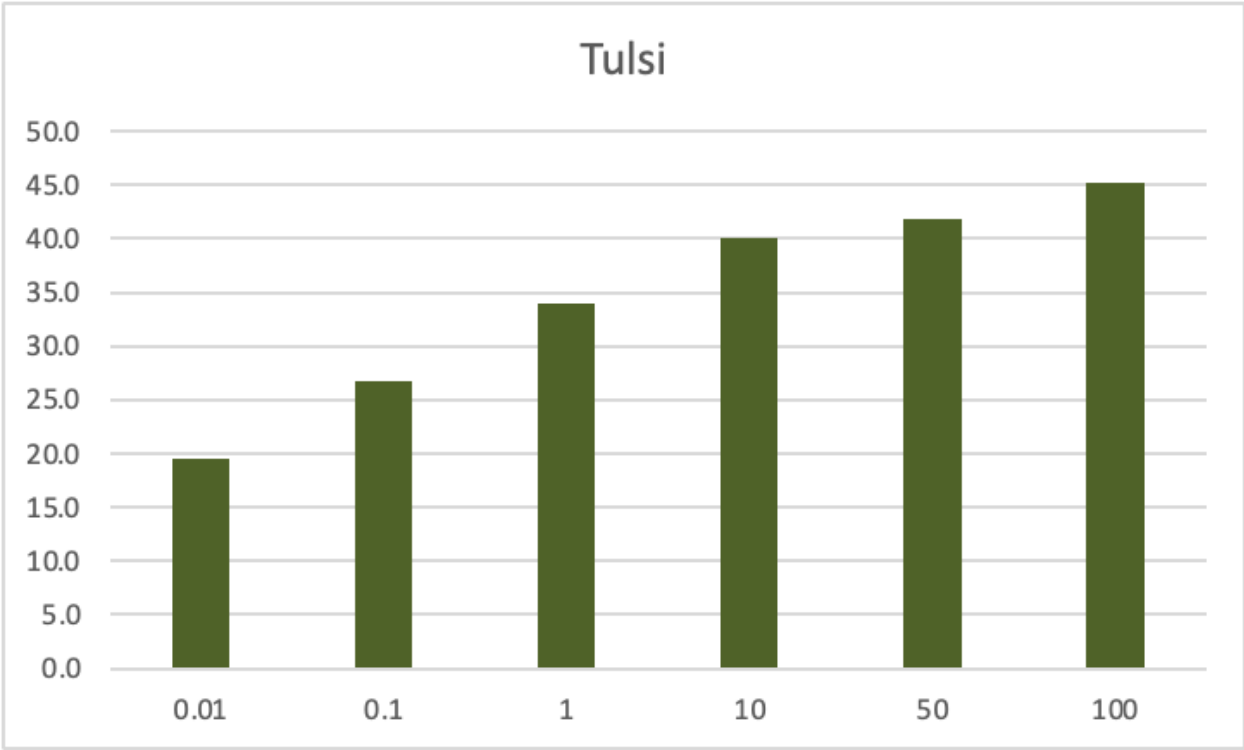
7.Statistical analysis MTT Data were analyzed by using Microsoft office Excel Software.

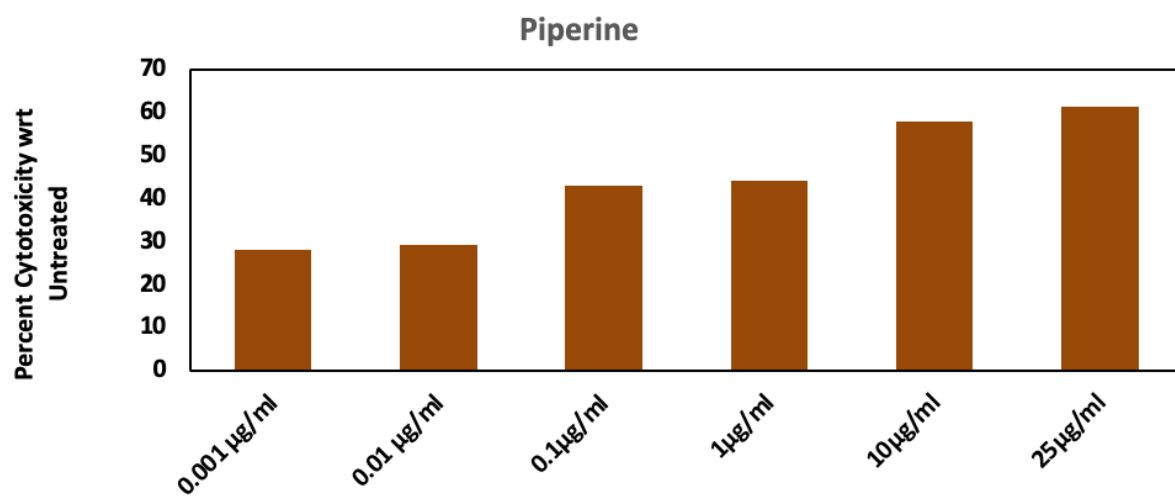
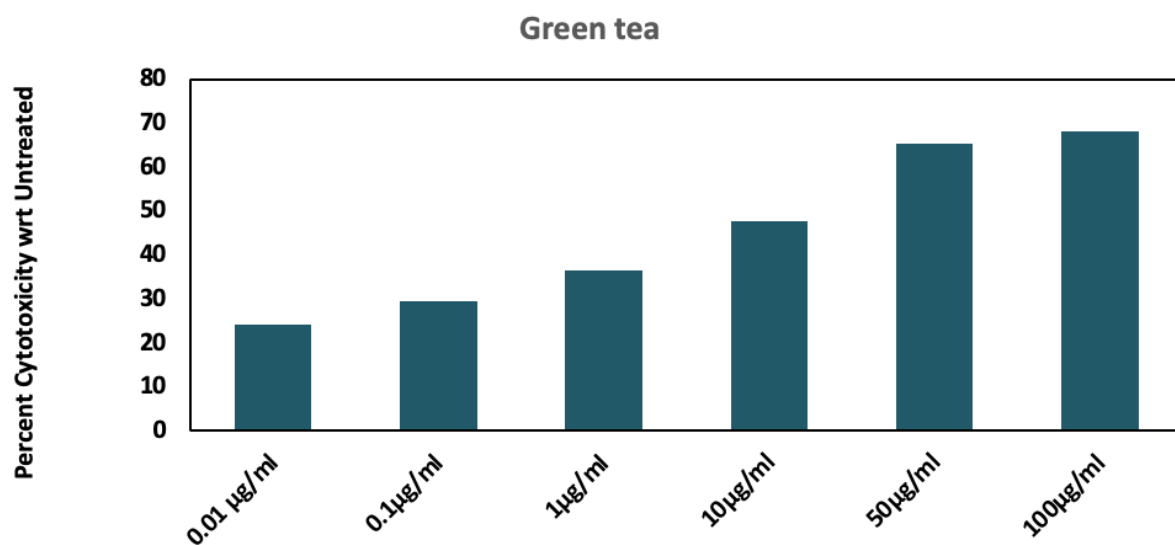
Results

Cytotoxicity Assay: During my experiment I found that Doxorubicin (a drug used in chemotherapy) showed that as the concentration of doxorubicin increased, the cytotoxicity also increased (from 11.7% at 0.1 μ M to 69.2% at 50 μ M). The IC₅₀ value is 9.391 μ M, which is the concentration of doxorubicin that causes 50% cytotoxicity. The compounds Wheat Grass, Ginger, Tulsi, Garlic, Ashwagandha, Green Tea, Piperine, Turmeric it was observed that as the concentration of the substance increased, the % cytotoxicity increased.The IC₅₀ value is the concentration at which 50% cytotoxicity is observed for each substance. Some substances, such as Wheat Grass, Ginger, and Tulsi, have IC₅₀ values greater than 100 μ g/ml, indicating that higher concentrations are required to reach 50% cytotoxicity.The substances Garlic, Ashwagandha, Green Tea, Piperine, and Turmeric have IC₅₀ values less than 100 μ g/ml, suggesting relatively lower concentrations are needed to induce 50% cytotoxicity.

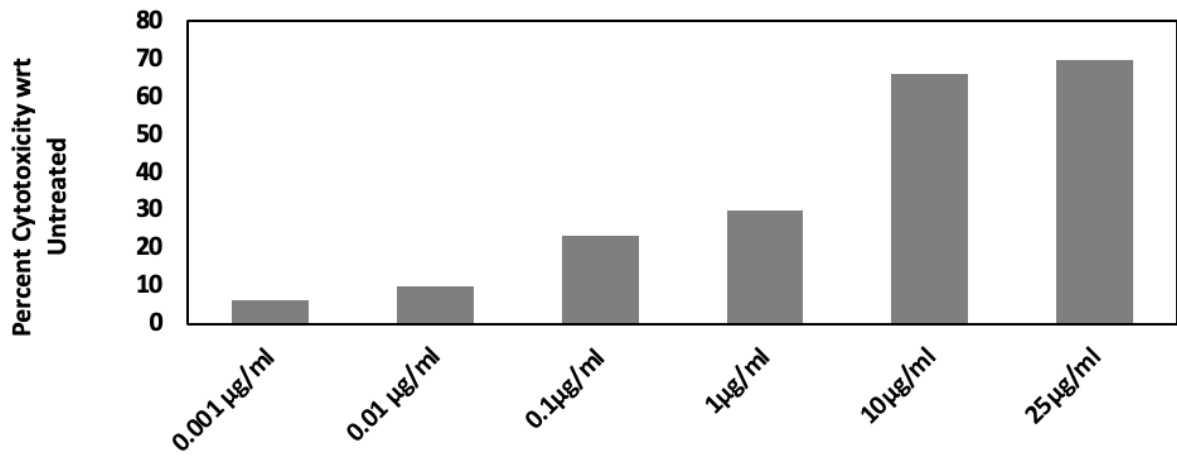






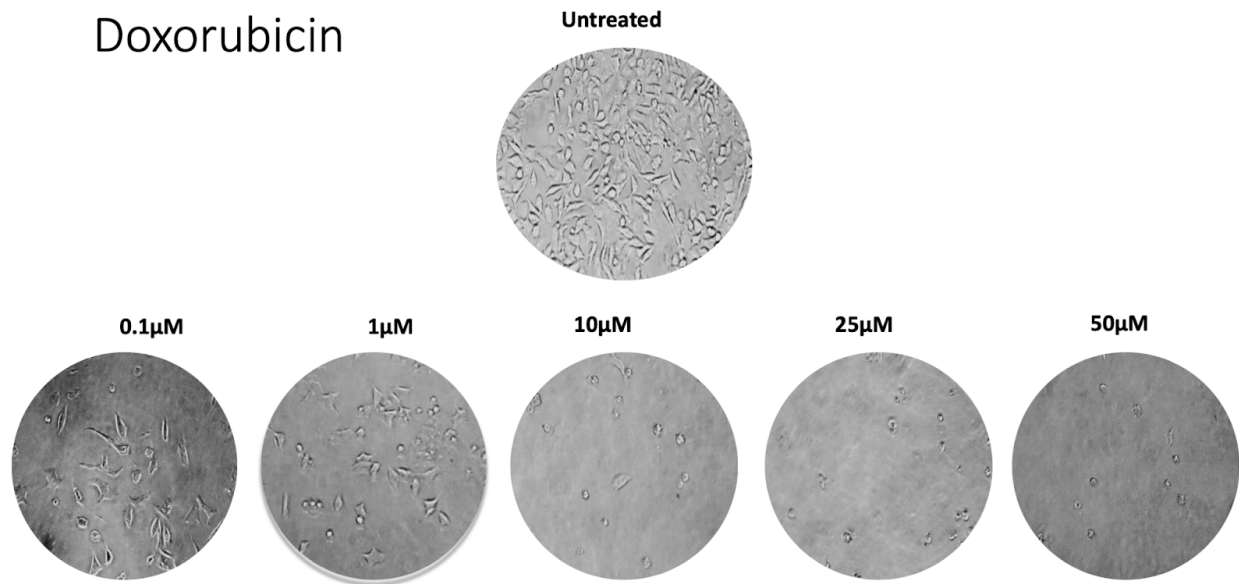


Turmeric

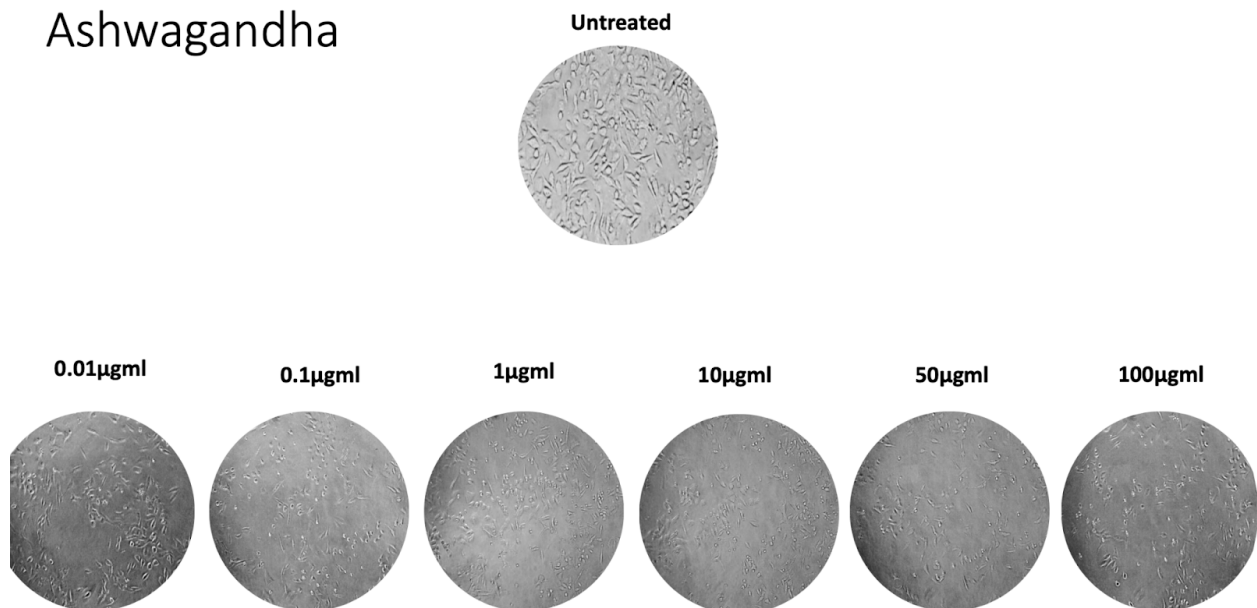


Evaluation of in-vitro cytotoxic potential of herbal products in human Lung cancer cell line (A549) by antioxidant assay: All the spices that were used from ginger to turmeric showed a good amount of percentage inhibition for the cancer cells. Some spices, such as garlic, green tea extract, and wheatgrass extract, demonstrate relatively higher cytotoxicity, with percent inhibition reaching as high as 85% or more at certain concentrations. These substances may have potential as cytotoxic agents for further investigation.

Doxorubicin

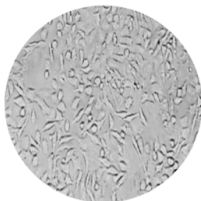


Ashwagandha



Garlic

Untreated



0.01µgml



0.1µgml



1µgml



10µgml



50µgml

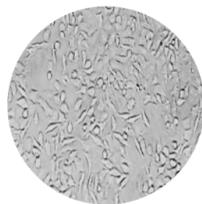


100µgml

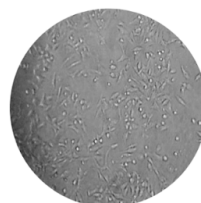


Ginger

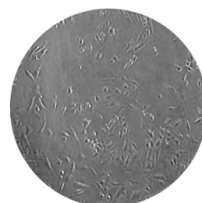
Untreated



0.01µgml



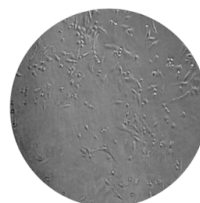
0.1µgml



1µgml



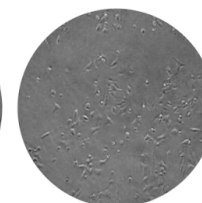
10µgml



50µgml

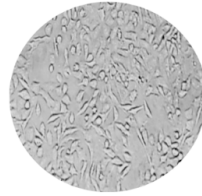


100µgml



Green Tea

Untreated



0.01 μ g/ml



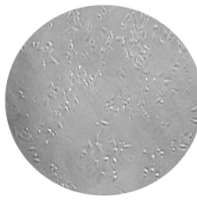
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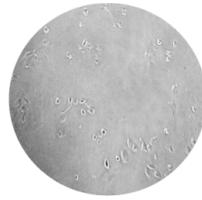
1 μ g/ml



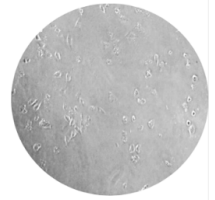
10 μ g/ml



50 μ g/ml

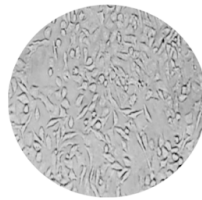


100 μ g/ml



Piperine

Untreated



0.001 μ g/ml



0.01 μ g/ml



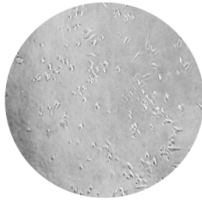
0.1 μ g/ml



1 μ g/ml



10 μ g/ml

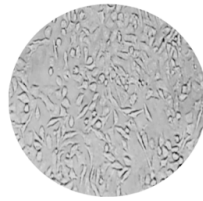


25 μ g/ml



Tulsi

Untreated



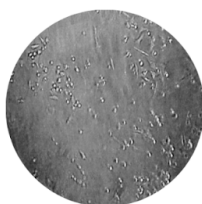
0.01 μ g/ml



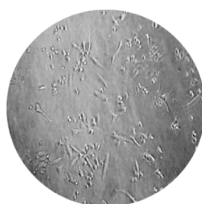
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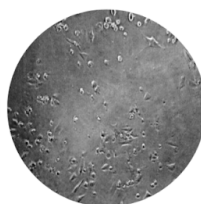
1 μ g/ml



10 μ g/ml



50 μ g/ml

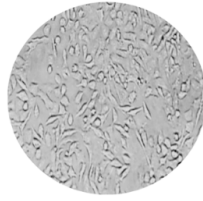


100 μ g/ml



Turmeric

Untreated



0.001 μ g/ml



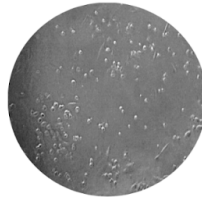
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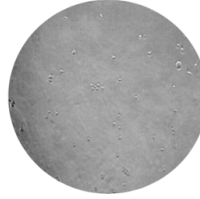
0.1 μ g/ml



1 μ g/ml



10 μ g/ml

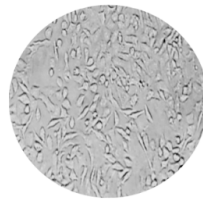


25 μ g/ml



Wheat Grass

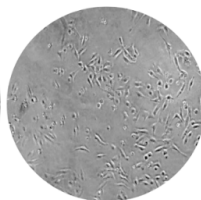
Untreated



0.01 μ g/ml



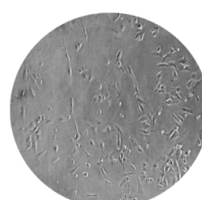
0.1 μ g/ml



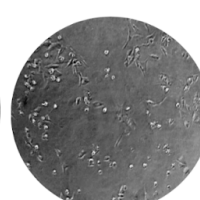
1 μ g/ml



10 μ g/ml



50 μ g/ml



100 μ g/ml



Conclusions

Because of the efficacy of each of the spices used, it is quite evident that spices show antiinflammatory effects. We believe that spices may play a crucial role in suppressing inflammation and preventing and treating cancer and other chronic diseases. The lower cancer incidence in countries with regular spice consumption supports the potential anti cancer properties of these dietary components. We predict relevant concentrations can not only reduce the spread of cancer but also can have a major control on the occurrence of chronic disorders.

Targeting inflammatory pathways may offer opportunities for both the prevention and treatment of cancer and other chronic diseases.

References

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