CHARACTERIZATION OF *PHYSALIS LAGASCAE* FOR ITS *IN VITRO* ANTI-CANCER ACTIVITY

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INTRODUCTION

1. INTRODUCTION

IMPORTANCE OF MEDICINAL PLANTS AND HERBS

The term “medicinal plant” include various types of plants used in herbalism ("herbology" or "herbal medicine"). It is the use of plants for medicinal purposes, and the study of such uses.The word “herb” has been derived from the Latin word, *“herba”* and an old French word *“herbe”*. Now a days, herb refers to any part of the plant like fruit, seed, stem, bark, flower, leaf, stigma or a root, as well as a non-woody plant. Earlier, the term “herb” was only applied to non-woody plants, including those that come from trees and shrubs. These medicinal plants are also used as food, flavonoid, medicine or perfume and also in certain spiritual activities.

Plants have been used for medicinal purposes long before prehistoric period. Ancient Unani manuscripts Egyptian papyrus and Chinese writings described the use of herbs. Evidence exist that Unani Hakims, Indian Vaids and European and Mediterranean cultures were using herbs for over 4000 years as medicine. Indigenous cultures such as Rome, Egypt, Iran, Africa and America used herbs in their healing rituals, while other developed traditional medical systems such as Unani, Ayurveda and Chinese Medicine in which herbal therapies were used systematically.

Traditional systems of medicine continue to be widely practised on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Among ancient civilisations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and

aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been codified in AYUSH systems in INDIA. Ayurveda, Unani, Siddha and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda and Unani Medicine are most developed and widely practised in India.

Recently, WHO (World Health Organization) estimated that 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care needs. According to WHO, around 21,000 plant species have the potential for being used as medicinal plants?

As per data available over three-quarters of the world population relies mainly on plants and plant extracts for their health care needs. More than 30% of the entire plant species, at one time or other were used for medicinal purposes. It has been estimated, that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as India and China, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world. These countries provide two third of the plants used in modern system of medicine and the health care system of rural population depend on indigenous systems of medicine. Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are in sync with nature, which is the biggest advantage. The golden fact is that, use of herbal treatments is independent of any age groups and the sexes.

The ancient scholars only believed that herbs are only solutions to cure a number of health related problems and diseases. They conducted thorough study about the same, experimented to arrive at accurate conclusions about the efficacy of different herbs that have medicinal value. Most of the drugs, thus formulated, are

free of side effects or reactions. This is the reason why herbal treatment is growing in popularity across the globe. These herbs that have medicinal quality provide rational means for the treatment of many internal diseases, which are otherwise considered difficult to cure. Medicinal plants such as *Aloe, Tulsi, Neem, Turmeric* and *Ginger* cure several common ailments. These are considered as home remedies in many parts of the country. It is known fact that lots of consumers are using Basil (*Tulsi*) for making medicines, black tea, in *pooja* and other activities in their day to day life.

In several parts of the world many herbs are used to honour their kings showing it as a symbol of luck. Now, after finding the role of herbs in medicine, lots of consumers started the plantation of tulsi and other medicinal plants in their home gardens .Medicinal plants are considered as a rich resources of ingredients which can be used in drug development either pharmacopoeia, non- pharmacopoeia or synthetic drugs. A part from that, these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants are considered as important source of nutrition and as a result of that they are recommended for their therapeutic values. Some of these plants include ginger, green tea, walnuts, aloe, pepper and turmeric etc. Some plants and their derivatives are considered as important source for active ingredients which are used in aspirin and toothpaste etc.

Apart from the medicinal uses, herbs are also used in natural dye, pest control, food, perfume, tea and so on. In many countries different kinds of medicinal plants/ herbs are used to keep ants, flies, mice and flee away from homes and offices. Nowadays medicinal herbs are important sources for pharmaceutical manufacturing.

Recipes for the treatment of common ailments such as diarrhoea, constipation, hypertension, low sperm count, dysentery and weak penile erection,

piles, coated tongue, menstrual disorders, bronchial asthma, leucorrhoea and fevers are given by the traditional medicine practitioners very effectively .Over the past two decades, there has been a tremendous increase in the use of herbal medicine; however, there is still a significant lack of research data in this field. Therefore since 1999, WHO has published three volumes of the WHO monographs on selected medicinal plants.

Importance of some herbs with their medicinal values

- Herbs such as black pepper, cinnamon, myrrh, aloe, sandalwood, ginseng, red clover, burdock, bayberry, and safflower are used to heal wounds, sores and boils.

- Basil, Fennel, Chives, Cilantro, Apple Mint, Thyme, Golden Oregano, Variegated Lemon Balm, Rosemary, Variegated Sage are some important medicinal herbs and can be planted in kitchen garden. These herbs are easy to grow, look good, taste and smell amazing and many of them are magnets for bees and butterflies.

- Many herbs are used as blood purifiers to alter or change a long-standing condition by eliminating the metabolic toxins. These are also known as 'blood cleansers'. Certain herbs improve the immunity of the person, thereby reducing conditions such as fever.

- Some herbs are also having antibiotic properties. Turmeric is useful in inhibiting the growth of germs, harmful microbes and bacteria. Turmeric is widely used as a home remedy to heal cut and wounds.

- To reduce fever and the production of heat caused by the condition, certain antipyretic herbs such as *Chirayta*, black pepper, sandal wood and safflower are recommended by traditional Indian medicine practitioners.

- Sandalwood and Cinnamon are great astringents apart from being aromatic. Sandalwood is especially used in arresting the discharge of blood, mucus etc.

- Some herbs are used to neutralize the acid produced by the stomach. Herbs such as marshmallow root and leaf. They serve as antacids. The healthy gastric acid needed for proper digestion is retained by such herbs.

- Indian sages were known to have remedies from plants which act against poisons from animals and snake bites.

- Herbs like Cardamom and Coriander are renowned for their appetizing qualities. Other aromatic herbs such as peppermint, cloves and turmeric add a pleasant aroma to the food, thereby increasing the taste of the meal.

- Some herbs like aloe, sandalwood, turmeric, sheetraj hindi and khare khasak are commonly used as antiseptic and are very high in their medicinal values.

- Ginger and cloves are used in certain cough syrups. They are known for their expectorant property, which promotes the thinning and ejection of mucus from the lungs, trachea and bronchi. Eucalyptus, Cardamom, Wild cherry and cloves are also expectorants.

- Herbs such as Chamomile, Calamus, Ajwain, Basil, Cardamom, Chrysanthemum, Coriander, Fennel, Peppermint and Spearmint, Cinnamon, Ginger and Turmeric are helpful in promoting good blood circulation. Therefore, they are used as cardiac stimulants.

- Certain medicinal herbs have disinfectant property, which destroys disease causing germs. They also inhibit the growth of pathogenic microbes that cause communicable diseases.

- Herbal medicine practitioners recommend calmative herbs, which provide a soothing effect to the body. They are often used as sedatives.

- Certain aromatic plants such as Aloe, Golden seal, Barberry and Chirayata are used as mild tonics. The bitter taste of such plants reduces toxins in blood. They are helpful in destroying infection as well.

- Certain herbs are used as stimulants to increase the activity of a system or an organ, for example herbs like Cayenne (Lal Mirch, Myrrh, Camphor and Guggul.

A wide variety of herbs including Giloe, Golden seal, Aloe and Barberry are used as tonics. They can also be nutritive and rejuvenate a healthy as well as diseased individual.

CANCER

Cancer is the name given to a collection of related diseases. In all types of cancer, some of the body's cells begin to divide without stopping and spread into surrounding tissue. Cancer can start almost anywhere in the human body, which is made up of trillions of cells. Normally, human cells grow and divide to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place.

When cancer develops, however, this orderly process breaks down. As cells become more and more abnormal, old or damaged cells survive when they should die, and new cells form when they are not needed. These extra cells can divide

without stopping and may form growths called tumors. Many cancers form solid tumors, which are masses of tissue. Cancers of the blood, such as leukemias, generally do not form solid tumors .Cancerous tumors are malignant, which means they can spread into, or invade, nearby tissues. In addition, as these tumors grow, some cancer cells can break off and travel to distant places in the body through the blood or the lymph system and form new tumors far from the original tumor.

Unlike malignant tumors, benign tumors do not spread into, or invade, nearby tissues. Benign tumors can sometimes be quite large, however. When removed, they usually don't grow back, whereas malignant tumors sometimes do. Unlike most benign tumors elsewhere in the body, benign brain tumors can be life threatening.

Differences between Cancer Cells and Normal Cells

Cancer cells differ from normal cells in many ways that allow them to grow out of control and become invasive. One important difference is that cancer cells are less specialized than normal cells. That is, whereas normal cells mature into very distinct cell types with specific functions, cancer cells do not. This is one reason that, unlike normal cells, cancer cells continue to divide without stopping.

In addition, cancer cells are able to ignore signals that normally tell cells to stop dividing or that begin a process known as programmed cell death, or apoptosis, which the body uses to get rid of unneeded cells.

Cancer cells may be able to influence the normal cells, molecules, and blood vessels that surround and feed a tumor—an area known as the microenvironment. For instance, cancer cells can induce nearby normal cells to form blood vessels that supply tumors with oxygen and nutrients, which they need to grow. These blood vessels also remove waste products from tumors.

Cancer cells are also often able to evade the immune system, a network of organs, tissues, and specialized cells that protects the body from infections and other conditions. Although the immune system normally removes damaged or abnormal cells from the body, some cancer cells are able to "hide" from the immune system. Tumors can also use the immune system to stay alive and grow. For example, with the help of certain immune system cells that normally prevent a runaway immune response, cancer cells can actually keep the immune system from killing cancer cells.

MECHANISM OF CANCER ARISES

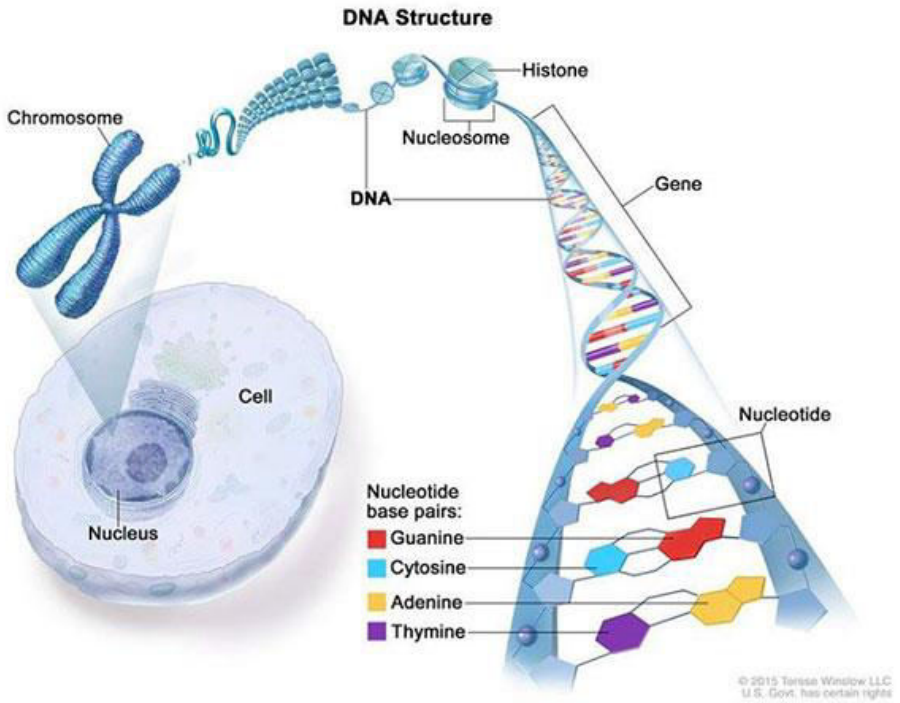


Fig. 1: Mechanism of cancer arises

Cancer is caused by certain changes to genes, the basic physical units of inheritance. Genes are arranged in long strands of tightly packed DNA called chromosomes.

Credit: Terese Winslow

Cancer is a genetic disease—that is, it is caused by changes to genes that control the way our cells function, especially how they grow and divide. Genetic changes that cause cancer can be inherited from our parents. They can also arise during a person's lifetime as a result of errors that occur as cells divide or because of damage to DNA caused by certain environmental exposures. Cancer-causing environmental exposures include substances, such as the chemicals in tobacco smoke, and radiation, such as ultraviolet rays from the sun. (Our Cancer Causes and Prevention section has more information.)

Each person's cancer has a unique combination of genetic changes. As the cancer continues to grow, additional changes will occur. Even within the same tumor, different cells may have different genetic changes.

In general, cancer cells have more genetic changes, such as mutations in DNA, than normal cells. Some of these changes may have nothing to do with the cancer; they may be the result of the cancer, rather than its cause.

"Drivers" of Cancer

The genetic changes that contribute to cancer tend to affect three main types of genes—proto-oncogenes, tumor suppressor genes, and DNA repair genes. These changes are sometimes called "drivers" of cancer. Proto-oncogenes are involved in normal cell growth and division. However, when these genes are altered in certain ways or are more active than normal, they may become cancer-causing genes (or oncogenes), allowing cells to grow and survive when they should not. Tumor suppressor genes are also involved in controlling cell growth and division. Cells with certain alterations in tumor suppressor genes may divide in an uncontrolled manner.

DNA repair genes are involved in fixing damaged DNA. Cells with mutations in these genes tend to develop additional mutations in other genes. Together, these mutations may cause the cells to become cancerous. As scientists have learned more about the molecular changes that lead to cancer, they have found that certain mutations commonly occur in many types of cancer. Because of this, cancers are sometimes characterized by the types of genetic alterations that are believed to be driving them, not just by where they develop in the body and how the cancer cells look under the microscope.

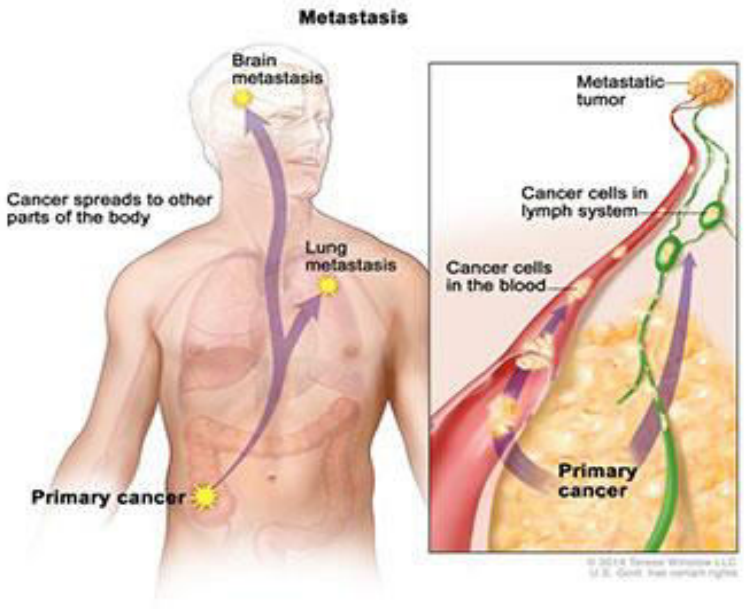


Fig. 2: Metastasis

In metastasis, cancer cells break away from where they first formed (primary cancer), travel through the blood or lymph system, and form new tumors (metastatic tumors) in other parts of the body. The metastatic tumor is the same type of cancer as the primary tumor.

A cancer that has spread from the place where it first started to another place in the body is called metastatic cancer. The process by which cancer cells spread to other parts of the body is called metastasis. Metastatic cancer has the same name and

the same type of cancer cells as the original, or primary, cancer. For example, breast cancer that spreads to and forms a metastatic tumor in the lung is metastatic breast cancer, not lung cancer. Under a microscope, metastatic cancer cells generally look the same as cells of the original cancer. Moreover, metastatic cancer cells and cells of the original cancer usually have some molecular features in common, such as the presence of specific chromosome changes.

Treatment may help prolong the lives of some people with metastatic cancer. In general, though, the primary goal of treatments for metastatic cancer is to control the growth of the cancer or to relieve symptoms caused by it. Metastatic tumors can cause severe damage to how the body functions, and most people who die of cancer die of metastatic disease.

Tissue Changes that Are Not Cancer

Not every change in the body's tissues is cancer. Some tissue changes may develop into cancer if they are not treated, however. Here are some examples of tissue changes that are not cancer but, in some cases, are monitored:

Hyperplasia occurs when cells within a tissue divide faster than normal and extra cells build up, or proliferate. However, the cells and the way the tissue is organized look normal under a microscope. Hyperplasia can be caused by several factors or conditions, including chronic irritation.

Dysplasia is a more serious condition than hyperplasia. In dysplasia, there is also a buildup of extra cells. But the cells look abnormal and there are changes in how the tissue is organized. In general, the more abnormal the cells and tissue look, the greater the chance that cancer will form. Some types of dysplasia may need to be monitored or treated. An example of dysplasia is an abnormal mole (called a

dysplastic nevus) that forms on the skin. A dysplastic nevus can turn into melanoma, although most do not.

An even more serious condition is carcinoma in situ. Although it is sometimes called cancer, carcinoma in situ is not cancer because the abnormal cells do not spread beyond the original tissue. That is, they do not invade nearby tissue the way that cancer cells do. But, because some carcinomas in situ may become cancer, they are usually treated.

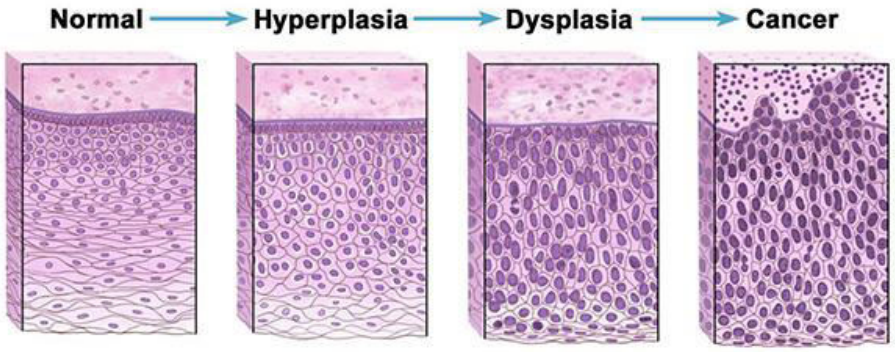


Fig. 3: Tissue changes of cancer

Normal cells may become cancer cells. Before cancer cells form in tissues of the body, the cells go through abnormal changes called hyperplasia and dysplasia. In hyperplasia, there is an increase in the number of cells in an organ or tissue that appear normal under a microscope. In dysplasia, the cells look abnormal under a microscope but are not cancer. Hyperplasia and dysplasia may or may not become cancer.

Types of Cancer

There are more than 100 types of cancer. Types of cancer are usually named for the organs or tissues where the cancers form. For example, lung cancer starts in cells of the lung, and brain cancer starts in cells of the brain. Cancers also may be

described by the type of cell that formed them, such as an epithelial cell or a squamous cell. Here are some categories of cancers that begin in specific types of cells:

Carcinoma

Carcinomas are the most common type of cancer. They are formed by epithelial cells, which are the cells that cover the inside and outside surfaces of the body. There are many types of epithelial cells, which often have a column-like shape when viewed under a microscope.

Carcinomas that begin in different epithelial cell types have specific names:

Adenocarcinoma is a cancer that forms in epithelial cells that produce fluids or mucus. Tissues with this type of epithelial cell are sometimes called glandular tissues. Most cancers of the breast, colon, and prostate are adenocarcinomas. Basal cell carcinoma is a cancer that begins in the lower or basal (base) layer of the epidermis, which is a person's outer layer of skin.

Squamous cell carcinoma is a cancer that forms in squamous cells, which are epithelial cells that lie just beneath the outer surface of the skin. Squamous cells also line many other organs, including the stomach, intestines, lungs, bladder, and kidneys. Squamous cells look flat, like fish scales, when viewed under a microscope. Squamous cell carcinomas are sometimes called epidermis carcinomas.

Transitional cell carcinoma is a cancer that forms in a type of epithelial tissue called transitional epithelium, or urothelium. This tissue, which is made up of many layers of epithelial cells that can get bigger and smaller, is found in the linings of the bladder, ureters, and part of the kidneys (renal pelvis), and a few other organs. Some cancers of the bladder, ureters, and kidneys are transitional cell carcinomas.

Sarcoma

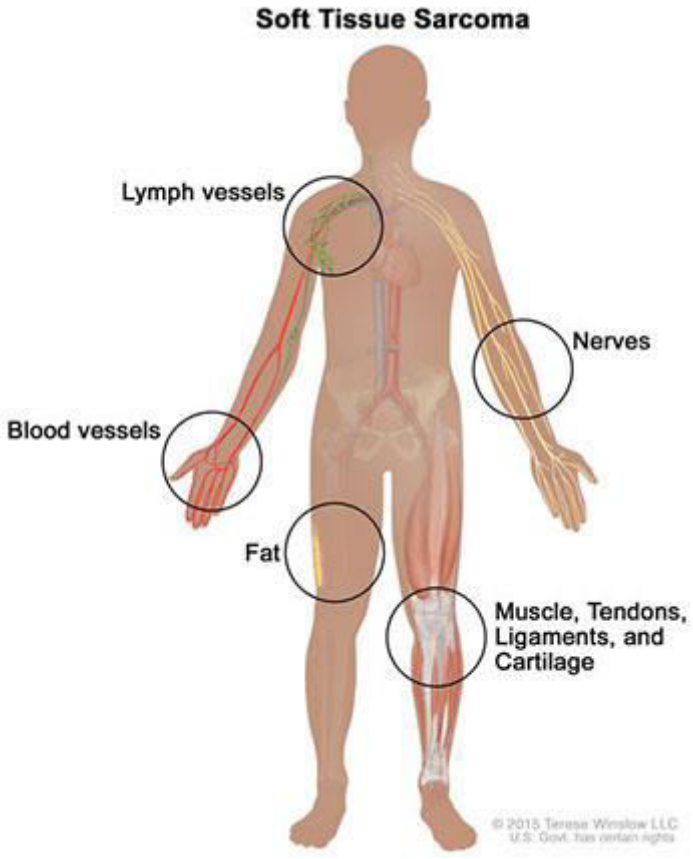


Fig. 4: Soft tissue sarcoma

Soft tissue sarcoma forms in soft tissues of the body, including muscle, tendons, fat, blood vessels, lymph vessels, nerves, and tissue around joints.

Sarcomas are cancers that form in bone and soft tissues, including muscle, fat, blood vessels, lymph vessels, and fibrous tissue (such as tendons and ligaments).

Osteosarcoma is the most common cancer of bone. The most common types of soft tissue sarcoma are leiomyosarcoma, Kaposi sarcoma, malignant fibrous histiocytoma, liposarcoma, and dermatofibrosarcoma protuberans.

Our page on soft tissue sarcoma has more information.

Leukemia

Cancers that begin in the blood-forming tissue of the bone marrow are called leukemias. These cancers do not form solid tumors. Instead, large numbers of abnormal white blood cells (leukemia cells and leukemic blast cells) build up in the blood and bone marrow, crowding out normal blood cells. The low level of normal blood cells can make it harder for the body to get oxygen to its tissues, control bleeding, or fight infections.

There are four common types of leukemia, which are grouped based on how quickly the disease gets worse (acute or chronic) and on the type of blood cell the cancer starts in (lymphoblastic or myeloid).

Lymphoma

Lymphoma is cancer that begins in lymphocytes (T cells or B cells). These are disease-fighting white blood cells that are part of the immune system. In lymphoma, abnormal lymphocytes build up in lymph nodes and lymph vessels, as well as in other organs of the body.

There are two main types of lymphoma:

Hodgkin lymphoma – People with this disease have abnormal lymphocytes that are called Reed-Sternberg cells. These cells usually form from B cells.

Non-Hodgkin lymphoma – This is a large group of cancers that start in lymphocytes. The cancers can grow quickly or slowly and can form from B cells or T cells.

Multiple Myeloma

Multiple myeloma is cancer that begins in plasma cells, another type of immune cell. The abnormal plasma cells, called myeloma cells, build up in the bone marrow and form tumors in bones all through the body. Multiple myeloma is also called plasma cell myeloma and Kahler disease.

Melanoma

Melanoma is cancer that begins in cells that become melanocytes, which are specialized cells that make melanin (the pigment that gives skin its color). Most melanomas form on the skin, but melanomas can also form in other pigmented tissues, such as the eye.

Brain and Spinal Cord Tumors

There are different types of brain and spinal cord tumors. These tumors are named based on the type of cell in which they formed and where the tumor first formed in the central nervous system. For example, an astrocytic tumor begins in star-shaped brain cells called astrocytes, which help keep nerve cells healthy. Brain tumors can be benign (not cancer) or malignant (cancer).

Other Types of Tumors

Germ Cell Tumors

Germ cell tumors are a type of tumor that begins in the cells that give rise to sperm or eggs. These tumors can occur almost anywhere in the body and can be either benign or malignant.

Neuroendocrine Tumors

Neuroendocrine tumors form from cells that release hormones into the blood in response to a signal from the nervous system. These tumors, which may make higher-than-normal amounts of hormones, can cause many different symptoms. Neuroendocrine tumors may be benign or malignant.

Carcinoid Tumors

Carcinoid tumors are a type of neuroendocrine tumor. They are slow-growing tumors that are usually found in the gastrointestinal system (most often in the rectum and small intestine). Carcinoid tumors may spread to the liver or other sites in the body, and they may secrete substances such as serotonin or prostaglandins, causing carcinoid syndrome.

PATHOPHYSIOLOGY

Cancer is disease of regulation of tissue growth. In this disease, the cells of the body display uncontrolled growth, invasion that intrudes and destroys adjacent tissues and spreads to other body locations. In order for a normal cell to transform into a cancer cell, genes which regulate cell growth and differentiation must be alter cellular transformation and Derangement theory. In this theory, exposure of normal cells to some etiologic agent may transform normal cells into cancer cells.

Failure of the Immune Response Theory. This theory conceptualizes that all individuals possess cancer cells but these cancer cells are NOT recognized by the immune system. Thus, cancer cells undergo destruction. Failure of the immune response system to kill or destroy the cancer cells leads to cancer.

Etiologic Factors or Carcinogens

- Viruses or Oncogenic Viruses. Prolonged and recurrent viral infections may lead to the breakdown of the immune system. The overwhelmed immune system may fail to destroy the cancer cells present in the body. The human papillomavirus (HPV) are particularly common cancer-causing virus which is well-known for causing genital warts and all cases of cervical cancer.
- Chemical carcinogens. These chemicals cause cell mutation or alter the cell enzymes and proteins.

Industrial Compounds

1. Vinyl chloride – plastic manufacture, asbestos factories, construction works
2. Polycyclic aromatic hydrocarbons
3. Fertilizers
4. Weed killers
5. Dyes – analine dyes (most commonly found in beauty shops and used at homes), hair bleach
6. Drugs – cytotoxic drugs, tar nicotine in tobacco, alcohol

Hormones

1. Estrogen
2. Diethystilbesterol (DES)

Foods, preservatives

1. Nitrites in bacon or smoked meat
2. Talc (polished rice, salami and chewing gum)
3. Food sweeteners
4. Nitrosomines (rubber baby nipples)
5. Aflatoxins (mold in nuts, grains, milk, cheese and peanut butter)
6. Polycyclic hydrocarbons
7. Physical agents

Radiation

1. From x-rays or radioactive isotopes
2. From sunlight or UV rays

Physical irritation or trauma

1. Pipe smoking
2. Multiple deliveries
3. Genetics

Risk Factors

- Older individuals
- Women are more prone to breast, uterine and cervical cancer
- Men are more prone prostate and lung cancer

- Urban dwellers
- Chemical factory workers
- Farmers
- Personnel of radiology department
- Family history

Mechanism of action of anticancer agents from pant source

Cancers are characterized by the dysregulation of cell signaling pathways at multiple steps. However, most current anticancer therapies involve the modulation of a single target. The lack of safety and high cost of monotargeted therapies have encouraged alternative approaches. Both natural compounds, extracted from plants or animals, and synthetic compounds, derived from natural prototype structures, are now being used as cancer therapeutics and as chemopreventive compounds. In this report we will review four major classes of plant-derived anti-cancer drugs.

DNA methylation pattern is essential in development and can be altered in human tumors. Tumor cells are characterized by specific genetic and epigenetic changes that promote uncontrolled cellular proliferation. Based on the rationale that hypermethylation-induced gene silencing could be uncovered by gene demethylation and reactivation, many efforts have been put in the identification and characterization of inhibitors of DNA methylation as tools to treat cancer. Several studies suggested that green tea possess chemopreventive and therapeutic potential against tumor cells. Much of the anticancer and/or cancer chemopreventive effects of green tea are mediated by its most abundant catechin, epigallocatechin-3-gallate (EGCG). EGCG has been shown to possess strong anti-proliferative and anti-tumor

effects both *in vitro* and in animal models. EGCG inhibited DNA methyltransferase activity with reactivation of epigenetically silenced tumor suppressor genes.

Chromatin acetylation is another major epigenetic modification that is regulated by the balanced action of histone acetyltransferases (HAT) and deacetylases (HDAC) (1). HDAC inhibitors (HDACi) reactivate epigenetically-silenced genes in cancer cells, triggering cell cycle arrest and apoptosis. HDACi can enhance the sensitivity to chemotherapy for cancers and inhibit angiogenesis. A number of natural and synthetic HDACi have shown an anti-proliferative activity on tumor cells. Recent evidence suggests that dietary constituents, such as the isothiocyanates found in cruciferous vegetables, can act as HDACi. Broccoli sprouts are a rich source of sulforaphane, an isothiocyanate that inhibits HDAC activity in human colon, prostate, and breast cancer cells. Isoflavones have also been shown to possess a strong antioxidant activity and to inhibit oxidative DNA damage. Pomiferin, a prenylated isoflavonoid is isolated from *Maclura pomifera*. Pomiferin has been shown to inhibit the activity of HDAC enzyme. It also exhibited growth inhibitory activity on five human tumor cell lines including

The HCT-15 colon tumor cell line.

Thymoquinone (TQ), the main bioactive component of the volatile oil of the black seed (*Nigella sativa*, Ranunculaceae family), is a pleiotropic agent targeting multiple signaling pathways in many patho-physiological conditions. Recent studies have documented the cancer cell specific effects of TQ affecting multiple targets suggesting a promising role as an anticancer agent.

Drugs that inhibit microtubule dynamics represent some of the most effective anticancer medications. These drugs bind to tubulin, and are classified as microtubule stabilizers or destabilizers. The two major classes of antimitotic drugs

used to treat cancer are the vinca alkaloids and the taxanes. Estramustine is another related drug that functions by binding to microtubules and MAPs and is used to treat prostate cancer. Vinca alkaloids were initially isolated from the pink periwinkle plant (*Catharantus roseus*; formerly vinca rosea Linn). The vinca alkaloids bind to β-tubulin near the GTP-binding site. Although the structures of the various vinca alkaloids vary only slightly, they have distinct niches as chemotherapeutic agents. Vincristine is most effective in treating leukemias, lymphomas and sarcomas. Vinblastine, which differs from vincristine only by substitution of a formyl for a methyl group, is effective in advanced testicular cancer, Hodgkin's disease and lymphoma. Vinorelbine is currently used to treat non-small cell lung cancer as a single agent or in combination with cisplatin. Vindesine is undergoing clinical trials, primarily for treatment of acute lymphocytic leukemia. Vinflunine, the newest member of the vinca alkaloid family is currently in clinical trials to test for activity against solid tumors. Another well-characterized drug-binding sites on tubulin/microtubules is the taxane-binding site. Taxanes are microtubule-targeting agents that bind to polymerized microtubules, stabilize the microtubule, and inhibit its disassembly leading ultimately to cell death by apoptosis. Paclitaxel (Taxol, Bristol-Meyers Squibb) was originally derived from the bark of the Pacific yew tree but can now, like docetaxel, be partially synthesized from the precursor 10-deactylbaccatin III, derived from needles of the European yew.

Inhibitors of topoisomerase I and II are anticancer drugs active in a variety of haematological and solid tumours. The plant-derived camptothecins (irinotecan, topotecan) act as inhibitors of topoisomerase I; the plant-derived epopodophyllotoxins (etoposide and teniposide) and the microbial-derived anthracyclines (e.g. doxorubicin, epirubicin) act as inhibitors of topoisomerase II. Despite the numerous categories of the plant-derived anti-cancer drugs, this report reviews only 4 classes of natural anticancer drugs: methyltransferase inhibitors,

HDAC inhibitors (HDACi), DNA damaging/pro-oxidant drugs and mitotic disrupters.

EGCG

EGCG has been shown to be an efficient scavenger of free radicals. There is evidence that the A-ring of EGCG may provide an antioxidant site. On the other hand, studies have suggested that the cell-killing activity of tea phenols may be related to their pro-oxidant activity since in the presence of the H_2O_2scavenger catalase, the EGCG-induced apoptosis was inhibited. Whereas EGCG has been shown to have strong antioxidant activity *in vitro*, such activity has been demonstrated only in some *in vivo* experiments. Among smokers, green tea consumption decreased oxidative DNA damage measured by lower urinary level of 8-hydroxydeoxyguanosine.

EGCG has been shown to exert antiproliferative effects by blocking the activation of transcription factors AP-1 and NF-kB by direct inhibition of specific kinases such as JNK. EGCG can also inhibit cyclin-dependent kinases, leading to hypophosphorylated Rb protein form causing G0/G1 arrest.

EGCG has been reported to induce apoptosis in many cancer cell lines, including leukemia, stomach, pancreas, and breast. EGCG sensitizes prostate carcinoma cells to TRAIL-mediated apoptosis, and it reduces telomerase activity in small-cell lung carcinoma. Caspase 3 activity seems to be required for green tea-induced apoptosis. Green tea has been shown to inhibit carcinogenesis induced by UV light and chemical carcinogens in rodents, as well as spontaneous tumorigenesis in wild-type and genetically modified mice. The drug was able to inhibit cancer growth and invasion in a xenograft mouse model with pancreatic cancer via up-regulation of caspase 3 activity and $p21^{WAF1}$ expression. EGCG was shown to have

demethylating activity by inhibiting methyltransferase and to elevate the transcription of tumor suppressor genes, an effect that can be further enhanced by the presence of HDACi.

Several studies have reported that EGCG inhibits the formation of new blood vessels by blocking VEGF expression in head and neck, breast, and colon cancer cells. In the TAMP mouse model, the expression of VEGF and matrix metalloproteases and p-ERKs 1 and 2 decreased when mice consumed green tea extract, and there were only low side-effects.Many case-control studies have shown that subjects who consume large amounts of tea had a lower risk of gastric, esophageal and breast cancer. A recent encouraging study reported that among patients consuming 600mg green tea catechins daily within one year, there was a remarkable 90% reduction in the rate of high-grade-PIN-positive men developing prostate cancer. EGCG is currently tested in phase I pharmacokinetic study to determine its systemic availability after single oral dose administration. This clinical study is the first to show that chemicals in green tea can increase detoxification enzymes (glutathione S-transferases) in humans. Clinical trials of green tea products, especially in prostate cancer patients have yielded encouraging results.

Interestingly, investigating the pharmacogenetics of EGCG revealed that mice are very similar to humans in terms of enzymatic ability to conjugate tea catechins. Because the levels of tea consumption are lower than those used in animal cancer chemoprevention, the amount of the tea phenols that reaches the target tissues is a limiting factor. Furthermore, there is no doubt that the involvement of EGCG pro-oxidation may differ *in vivo* where anti-oxidative capacity is much higher and oxygen partial pressure is much lower than that in cell culture medium. Nevertheless, it is expected that cancer can be prevented by consuming moderate

levels of tea especially for the oral cavity and the intestinal tract, and this concept has to be further tested in intervention human studies.

ANTIOXIDENTS

Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions. To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes (e.g., catalase and superoxide dismutase), produced internally, or the dietary antioxidants vitamin C, and vitamin E.

The term "antioxidant" is mostly used for two entirely different groups of substances: industrial chemicals that are added to products to prevent oxidation, and naturally occurring compounds that are present in foods and tissue. The former, industrial antioxidants, have diverse uses: acting as preservatives in food and cosmetics, and being oxidation-inhibitors in fuels.

Importantly, antioxidant dietary supplements have not yet been shown to improve health in humans, or to be effective at preventing disease. Supplements of beta-carotene, vitamin A, and vitamin E have no positive effect on mortality rate or cancer risk. Additionally, supplementation with selenium or vitamin E do not reduce the risk of cardiovascular disease.

Although certain levels of antioxidant vitamins in the diet are required for good health, there is still considerable debate on whether antioxidant-rich foods or supplements have anti-disease activity. Moreover, if they are actually beneficial, it is unknown which antioxidants are health-promoting in the diet and in what amounts beyond typical dietary intake. Some authors dispute the hypothesis that antioxidant

vitamins could prevent chronic diseases, and others maintain such that hypothesis is unproven and misguided. Polyphenols, which often have antioxidant properties in vitro, are not necessarily antioxidants in vivo due to extensive metabolism following digestion.

In many polyphenols the catechol group acts as an electron acceptor and is therefore responsible for the antioxidant activity. However, this catechol group undergoes extensive metabolism upon uptake in the human body, for example by catechol-O-methyl transferase, and is therefore no longer able to act as an electron acceptor. Many polyphenols may have non-antioxidant roles in minute concentrations that affect cell-to-cell signaling, receptor sensitivity, inflammatory enzyme activity or gene regulation. Although dietary antioxidants have been investigated for potential effects on neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis

IMPORTANCE OF ANTIOXIDENTS

Antioxidants are seemingly magical nutrients that can repair cell damage that happens in all our bodies over time -- including those of our cats. These nutrients occur naturally, but a body's supply needs an antioxidant boost from food. Common antioxidants include vitamin A, vitamin C, vitamin E and certain compounds called carotenoids (like lutein and beta-carotene)

As cells function normally in the body, they produce damaged molecules called free radicals, which are highly unstable and steal components from other cellular molecules, such as fat, protein, or DNA, thereby spreading the damage. This process, called peroxidation, continues in a chain reaction, and entire cells soon become damaged and die. Peroxidation is important because it helps the body destroy cells that have outlived their usefulness and kills germs and parasites.

However, peroxidation, when left unchecked, also destroys or damages healthy cells.

Antioxidants help prevent widespread cellular destruction by donating components to stabilize free radicals. More important, antioxidants return to the surface of the cell to stabilize, rather than damage, other cellular components.When there are not enough antioxidants to hold peroxidation in check, free radicals begin damaging healthy cells, which can lead to problems. For example, free radical damage to immune cells can lead to an increased risk of infections. Recent research has examined the benefits of certain antioxidants on the immune response of dogs and cats. The results of these studies indicated that antioxidants are important in helping dogs and cats maintain a healthy immune system. The research also showed each antioxidant benefits the immune system uniquely so one antioxidant at high levels is not as effective as a group of antioxidants acting together.

Nutritionally supporting the immune system may be especially critical for young animals. For example, the immune system in kittens is still developing at the time it is being challenged with vaccinations and exposure to disease-causing agents. With the addition of antioxidants, a proper kitten diet can aid in the development of a strong immune system to help maintain good health and protect against viruses, bacteria and parasites.

Antioxidants and Aging

Recent research has also examined the effect of aging on immune responses. The findings indicate that as dogs and cats age, immune cell responses may decline. Including antioxidants in the diet can reverse the age-related decrease in immune cell function. However, increased immune cell response is not always proportional

to the amount of vitamin E. Although feeding a diet containing 250 IU vitamin E/kg enhanced immune cell response in old cats, adding 500 IU/kg did not achieve the same beneficial effect. Many pet food labels include information about antioxidants, perhaps indicating that the product was formulated to contain this beneficial nutrient. Here are some common ingredients to look for, and how they may help your cat:

- Vitamin E Optimizes immune system's T-cell activation
- Lutein Optimizes immune system's B-cell activation and helps vaccine recognition
- Beta-carotene Optimizes types of cell present in the blood, increases antibody levels in the blood and helps vaccine recognition

Antioxidants may not prevent all health problems, but there is enough evidence to suggest that they promote good health. Since these nutrients don't change the flavour or texture of food much, advice to consume them should be easy for you and your cat to swallow.

ANTI OXIDENTS IN CANCER THERAPY

There are studies that show that low antioxidant status and increased oxidative stress are seen in cancer patients, even before oncology treatment starts. Patients with tongue carcinoma and found that the pre-treatment levels of plasma lipid peroxide and conjugated dines were Three-stage model of carcinogenesis and the level of carcinogenic effect. A multistage process such as cancer development is characterized by the cumulative action of multiple events occurring in a single cell and can be described by three stages: (1) initiation, (2) promotion, and (3) progression. ROS can act in all these stages of carcinogenesis. (1) Initiation stage

produces an altered cell followed by at least one round of DNA synthesis to fix the damage (eg, 8-OH-G) produced during the initiation. (2) The promotion stage is characterized by the clonal expansion of initiated cells by the induction of cell proliferation and/or inhibition of programmed cell death (apoptosis). (3) Progression stage involves cellular and molecular changes that occur from the preneoplastic to the neoplastic state, is irreversible and s characterized by accumulation of additional genetic damage, leading to the transition of the cell from benign to malignant, and is characterized by genetic instability and disruption of chromosome integrity. (Adapted from Vineis et al. [14].)

V. Fuchs-Tarlovsky / Nutrition 29 (2013) 15–21 significantly elevated in patients with carcinoma, as compared with controls (P ¼ 0.001). Significantly lowered levels of reduced glutathione, glutathione peroxidase, superoxide dismutase, and vitamin C and E were observed in cancer patients, when compared to control subjects. They concluded that increased levels of oxidative stress markers and decreased levels of antioxidants in carcinoma or the tongue suggest that oxidative stress markers play a significant role in pathophysiology of this cancer. In a study by Badajatia et al. , results showed that serum levels of vitamin C and E, whole blood levels of superoxide dismutase and glutathione peroxidase, and serum antioxidant activity were significantly lower ($P < 0.001$), whereas serum levels of MDA were significantly higher ($P < 0.001$) in patients than controls. Levels of all the biochemical parameters were correlated with the degree and severity of the disease. Results by Klarod et al. showed that when measuring serum levels of nonenzymatic antioxidants in lung cancer patients, retinol and lycopene levels were statically lower at early stages, whereas vitamin E, b-carotene, selenium, and zinc were even lower at advanced stages of the disease, and that serum selenium levels happened to be different when patients were divided according to their body mass index. Vitamin E or a-tocopherol has been defined as a radical-chain breaker, which,

due to its hydrophobic nature, operates in a lipid environment. The effects of a-tocopherol as an antioxidant are thus restricted to its direct effects in membranes and lipoprotein domains. Consequently, other definitions such as "secondary antioxidants" as an inhibitor of "enzymes that produce radicals," or activation of genes coding for "antioxidant enzymes," are confusing.

Tocopherols react with free radicals, notably peroxide radicals, and with singlet molecular oxygen ('O2), which is the base of its function as an antioxidant. RRR-a-Tocopherol is the major peroxyl radical scavenger in biological lipid phases such as membranes or low-density lipoproteins. In membranes such high reactivity is important because tocopherols react with lipid peroxyl radicals to yield a relatively stable lipid hydroperoxide, and the tocopheroxyl radical interrupts the radical chain reaction, thereby affording protection against lipid peroxidation Vitamin E is the major lipid soluble antioxidant protecting lipids against peroxidative damage. Studies indicate that oxidative cleavage of the phenyl side chain is a major metabolic pathway in humans, operative at saturated vitamin E plasma concentrations. In a study by Chitra et al, 2008 aimed to evaluate early and late effects of radiation and a-tocopherol on the secretion of saliva and on the selected saliva salivary parameters in oral cavity patients, the conclusion was that supplementation with a-tocopherol improved the salivary flow rate, thereby maintaining the salivary parameters. Vitamin C or L-ascorbic acid is water soluble and is present in its deprotonated state under most physiological conditions.

It is considered to be the most important antioxidant in extracellular fluids and has many cellular activities of an antioxidant nature as well. Vitamin C has been shown to scavenge superoxide, hydrogen, peroxide, hydroxyl radical, peroxyl radicals, and 'O2 efficiently. Ascorbic acid can also protect membranes against peroxidation by enhancing the activity of tocopherol, the chief lipid-soluble vitamin.

Although the value of vitamin C as a potential cancer treatment has been debated for decades, only one randomized clinical trial was found that evaluated vitamin C treatment concurrently with chemotherapy and reported on outcomes. In this study, a non-significant advantage was shown in objective response (complete response þ partial response), which was higher in the vitamin C supplemented group (60%) than in the placebo arm (33%). Additionally, although both groups had significant reductions in the sizes of the average lump diameter before and after treatment, the mean change was 3.53 0.73 in the vitamin C group versus 1.93 0.77 in the control group. Two recent studies have included vitamin C as part of an antioxidant mixture given concurrently with chemotherapy.

Pathak et al. Evaluated vitamins C, E, and b-carotene, whereas Weijl et al. evaluated vitamins C, E, and selenium. Weijl reported poor adherence to the supplemental regimen: 46% of all patients did not drink the beverage (placebo or antioxidant) throughout the entire study. Although the overall response rates were similar between the two groups (48% antioxidant group versus 44% control group), nine patients had complete response in the antioxidant group versus six patients in the placebo arm. A statistically significant correlation regarding improvement in toxicities was found between patients with the highest serum levels of antioxidant supplements and the lowest loss of high tone hearing after three cycles of chemotherapy (P ¼ 0.019). In the study by Pathak et al. , although none of the results achieved statistical significance, an advantage in overall response rates (37% versus 33%) and median survival (11 mo versus 9 mo) were seen for patients taking the antioxidant supplement

Much debate has arisen about whether antioxidant supplementation alters the efficacy of cancer chemotherapy. There is limited preliminary evidence by quality and sample size suggesting that certain antioxidant supplements may reduce adverse

reactions including neurotoxicity, asthenia, stomatitis/mucositis, and weight loss. Significant reductions in toxicity may alleviate dose-limiting toxicities so that more patients are able to complete prescribed chemotherapy regimens successfully, suggesting an improved therapeutic index. Because of the potential of the relationship between the reductions of dose-limiting toxicities allowing for full chemotherapy cycles and the subsequent potential for increased tumour. Response and/or survival, it is critical that future antioxidant/chemotherapy studies employ proper sample sizes and methodologies so that the results are of clear clinical relevance. Many studies indicate that antioxidant supplementation results in either increased survival times, increased tumor response, or both, as well as fewer toxicities than controls; in some of the last systematic reviews in this specific topic there is no evidence of antioxidant interference with chemotherapy mechanisms, with a possibility that antioxidants may even improve tumor response or patient survival. Combining these results with the potential for improvement of toxic side effects by antioxidants, additional strategies for further research on antioxidants and chemotherapy are now warranted

MECHANISM OF ACTION OF ANTIOXIDANTS

Antioxidants are molecules that inhibit or quench free radical reactions and delay or inhibit cellular damage. Though the antioxidant defenses are different from species to species, the presence of the antioxidant defense is universal. Antioxidants exists both in enzymatic and non-enzymatic forms in the intracellular and extracellular environment.

biochemical reactions, increased exposure to the environment, and higher levels of dietary xenobiotics result in the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS are responsible for the oxidative stress in different pathophysiological conditions. Cellular constituents of

our body are altered in oxidative stress conditions, resulting in various disease states. The oxidative stress can be effectively neutralized by enhancing cellular defenses in the form of antioxidants. Certain compounds act as in vivo antioxidants by raising the levels of endogenous antioxidant defenses. Expression of genes encoding the enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx) increases the level of endogenous antioxidants.

Antioxidants can be categorized in multiple ways. Based on their activity, they can be categorized as enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants work by breaking down and removing free radicals. The antioxidant enzymes convert dangerous oxidative products to hydrogen peroxide (H_2O_2) and then to water, in a multi-step process in presence of cofactors such as copper, zinc, manganese, and iron. Non-enzymatic antioxidants work by interrupting free radical chain reactions. Few examples of the non-enzymatic antioxidants are vitamin C, vitamin E, plant polyphenol, carotenoids, and glutathione.

The other way of categorizing the antioxidants is based on their solubility in the water or lipids. The antioxidants can be categorized as water-soluble and lipid-soluble antioxidants. The water-soluble antioxidants (e.g. vitamin C) are present in the cellular fluids such as cytosol, or cytoplasmic matrix. The lipid-soluble antioxidants (e.g. vitamin E, carotenoids, and lipoic acid) are predominantly located in cell membranes.

The antioxidants can also be categorized according to their size, the small-molecule antioxidants and large-molecule antioxidants. The small-molecule antioxidants neutralize the ROS in a process called radical scavenging and carry them away. The main antioxidants in this category are vitamin C, vitamin E, carotenoids, and glutathione (GSH). The large-molecule antioxidants are enzymes

(SOD, CAT, and GSHPx) and sacrificial proteins (albumin) that absorb ROS and prevent them from attacking other essential proteins.

To understand the mechanism of action of antioxidants, it is necessary to understand the generation of free radicals and their damaging reactions. This review elaborates the generation and damages that free radicals create, mechanism of action of the natural antioxidant compounds and assays for the evaluation of their antioxidant properties. The reaction mechanisms of the antioxidant assays are discussed. The scope of this article is limited to the natural antioxidants and the in vitro assays for evaluation of their antioxidant properties.

2. Generation of free radicals

The generation of ROSbegins with rapid uptake of oxygen, activation of NADPH oxidase, and the production of the superoxide anion radical ($O_2^{\cdot -}$, eqn (1)),

$$2O_2 + NADPH \xrightarrow{(oxidase)} 2O_2^{\cdot -} + NADP^+ + H^+$$

Table 1 List of the ROS

Symbol	Name
$^1O_2$	Singlet oxygen
$O_2^{\cdot -}$	Superoxide anion radical
$^{\cdot}OH$	Hydroxyl radical
$RO^{\cdot}$	Alkoxyl radical
$ROO^{\cdot}$	Peroxyl radical
H_2O_2	Hydrogen peroxide
LOOH	Lipid hydroperoxide

The $O_2^{\cdot -}$ is then rapidly converted to H_2O_2 (eqn (2)) by SOD

$$2O_2^{\cdot -} + 2H^+ \xrightarrow{(SOD)} H_2O_2 + O_2$$

These ROS can act by either of the two oxygen dependent mechanisms resulting in the destruction of the microorganism or other foreign matter. The reactive species can also be generated by the myeloperoxidase–halide–H_2O_2 system. The enzyme myeloperoxidase (MPO) is present in the neutrophil cytoplasmic granules. In presence of the chloride ion, which is ubiquitous, H_2O_2 is converted to hypochlorous (HOCl, eqn (3)), a potent oxidant and antimicrobial agent.[8]

$$Cl^- + H_2O_2 + H^+ \xrightarrow{(MPO)} HOCl + H_2O$$

ROS are also generated from $O_2^{\cdot -}$and H_2O_2 via 'respiratory burst' by Fenton (eqn (4)) and/or Haber–Weiss (eqn (5)) reactions.

$$H_2O_2 + Fe^{2+} \rightarrow {}^{\cdot}OH + OH^- + Fe^{3+}$$

$$O_2^{\cdot -} + H_2O_2 \rightarrow {}^{\cdot}OH + OH^- + O_2$$

The enzyme nitric oxide synthase produce reactive nitrogen species (RNS), such as nitric oxide ($NO^{\cdot}$) from arginine (eqn (6))

$$\text{L-Arg} + O_2 + \text{NADPH} \rightarrow NO^{\cdot} + \text{citrulline}$$

An inducible nitric oxide synthase (iNOS) is capable of continuously producing large amount of $NO^{\cdot}$, which act as a $O_2^{\cdot -}$quencher. The $NO^{\cdot}$ and $O_2^{\cdot -}$ react together to produce peroxynitrite ($ONOO^-$, eqn (7)), a very strong oxidant, hence, each can modulate the effects of other. Although neither $NO^{\cdot}$ nor $O_2^{\cdot -}$ is a strong oxidant, peroxynitrite is a potent and versatile oxidant that can attack a wide range of biological targets.

$$NO^{\cdot} + O_2^{\cdot -} \rightarrow ONOO^-$$

Peroxynitrite reacts with the aromatic amino acid residues in the enzyme resulting in the nitration of the aromatic amino acids. Such a change in the aminoacid residue can result in the enzyme inactivation. However, nitric oxide is an important cytotoxic effector molecule in the defense against tumor cells, various protozoa, fungi, helminthes, and mycobacteria. The other sources of free radical reactions are cyclooxygenation, lipooxygenation, lipid peroxidation, metabolism of xenobiotics, and ultraviolet radiations

3. Damaging reactions of free radicals

ROS induced oxidative stress is associated with the chronic diseases such as cancer, coronary heart disease (CHD), and osteoporosis. Free radicals attack all major classes of biomolecules, mainly the polyunsaturated fatty acids (PUFA) of cell membranes. The oxidative damage of PUFA, known as lipid peroxidation is particularly destructive, because it proceeds as a self-perpetuating chain reaction.

The general process of lipid peroxidation can be envisaged as depicted bellow (eqn (8)–(11)), where LH is the target PUFA and $R^{\cdot}$ is the initializing, oxidizing radical. Oxidation of the PUFA generates a fatty acid radical ($L^{\cdot}$) (eqn (8)), which rapidly adds oxygen to form a fatty acid peroxyl radical ($LOO^{\cdot}$, eqn (9)). The peroxyl radicals are the carriers of the chain reactions. The peroxyl radicals can further oxidize PUFA molecules and initiate new chain reactions, producing lipid hydroperoxides (LOOH) (eqn (10)and (11)) that can break down to yet more radical species.

$$LH + R^{\cdot} \rightarrow L^{\cdot} + RH$$

$$L^{\cdot} + O_2 \rightarrow LOO^{\cdot}$$

$$LOO^{\cdot} + LH \rightarrow LOOH + L^{\cdot}$$

$$LOOH \rightarrow LO^{\cdot} + LOO^{\cdot} + \text{aldehydes}$$

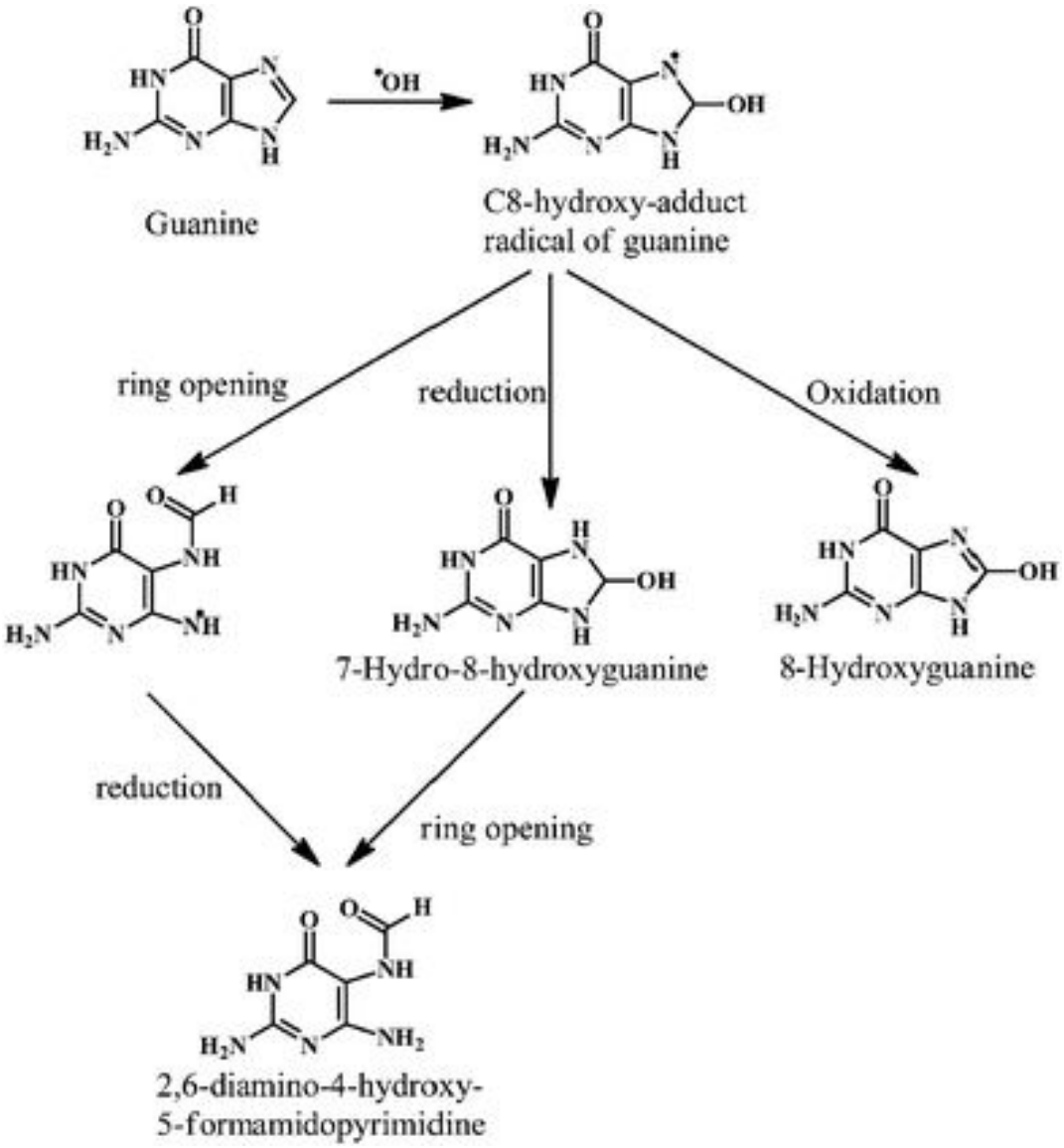
Lipid hydroperoxides always break down to aldehydes. Many of these aldehydes are biologically active compounds, which can diffuse from the original site of attack and spread the attack to the other parts of the cell. Lipid peroxidation has been widely associated with the tissue injuries and diseases.

Oxygen metabolism generates $^{\cdot}OH$, $O_2^{\cdot -}$, and the non-radical H_2O_2. The $^{\cdot}OH$ is highly reactive and reacts with biological molecules such as DNAs, proteins, and lipids, which results in the chemical modifications of these molecules. There are several research reports on the oxidative damage of DNA due to the $^{\cdot}OH$

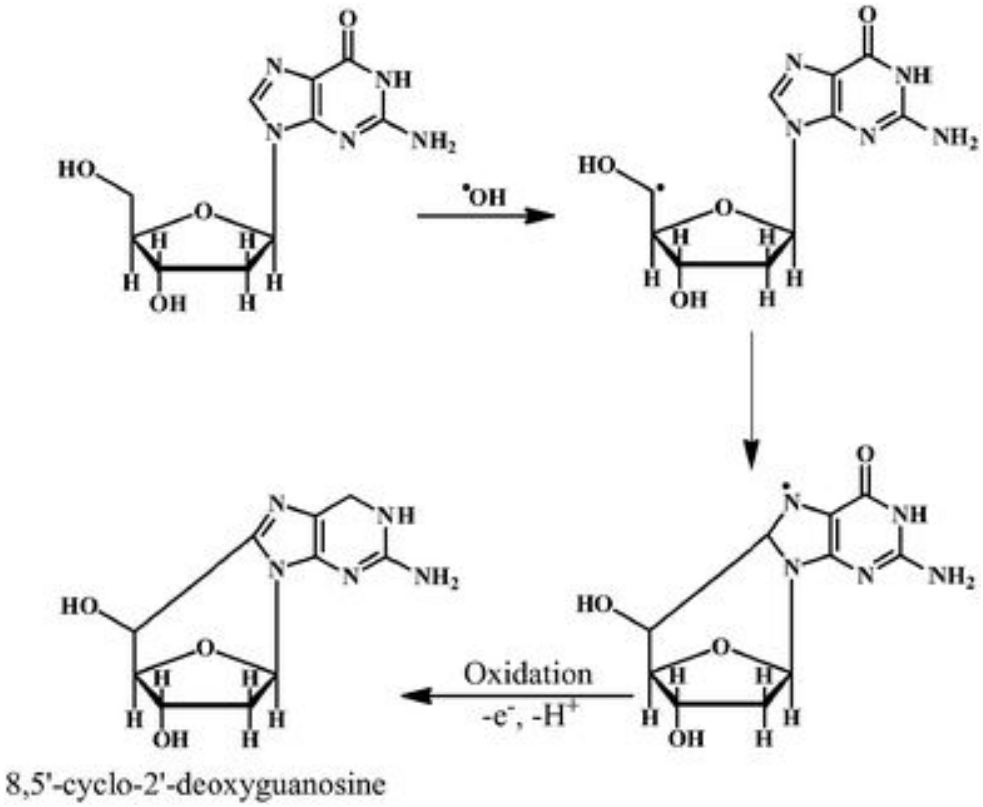
The $^{\cdot}OH$ reacts with the basepairs of DNA, resulting in the oxidative damage of the heterocyclic moiety and the sugar moiety in the oligonucleotides by a variety of mechanisms. This type of oxidative damage to DNA is highly correlated to the physiological conditions such as mutagenesis, carcinogenesis, and aging.The addition reactions yield OH-adduct radicals of DNA bases (Scheme 1), whereas the allyl radical of thymine and carbon-centered sugar radicals (Scheme 2) are formed from the abstraction reactions.

As shown in the Scheme 1, the $^{\cdot}OH$ reacts with the guanine of the DNA to produce the C-8-hydroxy-adduct radical of guanine, which is converted to the 2, 6-diamino-4-hydroxy-5-formamidopyrimidine upon reduction and ring opening reactions. However, the C-8-hydroxy-adduct radical of guanine is converted to the 8-hydroxyguanine upon oxidation reaction. The $^{\cdot}OH$ radical reacts with the heterocyclic moiety of the thymine and cytosine at C5- and C6-positions, resulting in the C5–OH and C6–OH adduct radicals, respectively. The oxidation reaction of these adduct radicals with water (followed by deprotonation) results in the formation

of the cytosine glycol and thymine glycol, respectively. Overall, the reactions of the ˙OH with the DNA bases result in the impaired dsDNA.



Scheme 1 Reaction of hydroxyl radical with guanine.



Scheme 2 Reaction of hydroxyl radical with the sugar moiety of DNA.

As shown in the Scheme 2, the ˙OH reacts with the sugar moiety of DNA by abstracting an H-atom from rom C5 carbon atom. One unique reaction of the C5′-centered radical of the sugar moiety in DNA is the addition to the C8-position of the purine ring in the same nucleoside (e.g. guanine). This intermolecular cyclization results in the formation of the 8, 5′-cyclopurine-2′-deoxynucleosides. The reactions of carbon-centered sugar radicals result in the DNA strand breaks and base-free sites by a variety of mechanisms.

Proteins are oxidatively damaged by the combined action of activated oxygen species and the trace metal ions such as $Fe^{2+}$and Cu^{2+}. The amino acids lysine, proline, histidine, and arginine have been found to be the most sensitive to oxidative damage. Recent studies indicate that, a wide range of residue modifications can occur including formation of peroxides, and carbonyls.Generation of the carbonyl

residue is a useful measure of oxidative damage to proteins. Thus, the oxidative damage to tissue results in the increased amount of oxidized protein. A detailed review by Cooke et al. provides important information on the oxidative DNA damage, mechanisms, mutations, and related diseases.

Low levels of antioxidants have been associated with the heart disease and cancer. Antioxidants provide protection against a number of disease processes such as aging, allergies, algesia, arthritis, asthma, atherosclerosis, autoimmune diseases, bronchopulmonary dyspepsia, and cancer. The other disorders to which antioxidants provide protection are cataract, cerebral ischemia, diabetes mellitus, eczema, gastrointestinal inflammatory diseases, and genetic disorders. Following section elaborates the mechanism of action of the radical scavenging activities of various natural antioxidant molecules.

2. LITERATURE REVIEW

Nephrotoxicityof *Physalislagascae*

Olagunjua *et al.* (2009) suggested a role for reactive oxygen metabolites as one of the postulated mechanisms in the pathogenesis of CCl4 nephrotoxicity. It has been hypothesized that physalis extract affords protection by impairing CCl4 mediated lipid peroxidation, through decreased production of free radical derivatives.

Gastric Ulcer of*Physalis lagascae*

A laboratory study was reported in which methanolic extract of *Physalis minima* leaves was investigated on ethanol induced ulcer models and pylorus ligation in wistar rats. The present study indicates that *Physalis minima* leaves extract have potential anti ulcer activity in both models. This results may further suggests that methanolic extract was found to possess anti-ulcerogenic as well as ulcer healing property, which might be anti-secretory activity

Anti-asthmaof *Physalislagascae*

The extract (100 and 200 mg/kg I.p) inhibited of albumin induced asthma by decreasing releasing of inflammatory mediators. *Physalis lagascaeroots* extract has potent anti-asthmatic activity. Its anti-asthmatic property probably acts via a reduction in inflammatory mediator's release. Thus the *Physalis lagascae*has significant anti-asthmatic property.

Diuretic effect of*Physalis lagascae*

Physalis was described to possess Diuretic effect petroleum ether extract of the Physalis minima in albino rats. The diuretic effect of the extract was evaluated

by measuring urine volume, sodium and potassium content. Methanolic Extract of *Physalis minima* produced notable diuretic effect which appeared to be comparable to that produced by the standard diuretic furosemide.

Anti-Microbial effect of *Physalis lagascae*

Methanol extract of *Physalis minima* exhibited Anti-Microbial activity, which was more active than that of standard linezolid, against a variety of clinically important microorganisms isolated from nearby hospitals in India and nonmutagenic up to 5 mg/plate in Ames test

Hepatoprotective effect of *Physalis lagascae*

As Physalis in rich in Flavonoids it is having hepatoprotective effect studies suggest that the aqueous and ethanol extracts prepared from the whole plant of these species were evaluated for their antihepatoma activity. The results conclude that plant possesses potent antihepatoma activity and its effect on apoptosis is associated with mitochondrial dysfunction.

3. AIM AND PLAN OF WORK

Medicinal plants have been in India for centuries as a therapeutic source for treating wide variety of ailments and have been found to be immense global importance

The family Solanacea consist important medicinal plants with wide range of pharmacological, biological activities and phytochemical constituents. The drug is used for a wide range of diseases which can be viewed from the literatures.

The selection of the plant physalis lagascae was made on the basis of its easy availability, therapeutic value and degree of research work which is not done.

PLAN OF WORK

The literature review reveals that the plant physalis lagascae was used to treat various ailments. However, there was a few pharmacological exploration was performed. Hence the present study undertaken for its scientific merits towards phytomedicine claim. The plan of work on the plant *physalis lagascae* was carried out as follow

1. Collection and identification of plant material

2. Preliminary phytochemical studies

a) Preparation of extract

b) Qualitative phytochemical analysis

c) Chromatographic studies

3. Pharmacological evaluation of the plant extract

a) Anticancer activity

4. PLANT PROFILE

Fig. 5: Images of *Physalis lagascae*

Fig. 6: Leaf of *Physalis lagascae*

Fig. 7: Whole plant of *Physalis lagascae*

Botanical name: *Physalis lagascae*

Common names

Little gooseberry, Wild Cape gooseberry, Ground Cherry, Sunberry

- **Hindi**: Rasbhari, Ban Tipariya, Chirpati
- **Marathi**: Chirboti, Nanvachivel, Ran-popti
- **Tamil**: Kupanti
- **Malayalam**: Notinotta
- **Telugu**: Kupanti
- **Kannada**: Gadde hannu
- **Bengali**: Bantepariya
- **Gujarati**: Popti;

Family: Solanacea

General

Erect to decumbent or prostrate, weak or occasionally ± robust, ± dichotomously much branched, annual herb, (0.05)0.1–1 m high (elsewhere said to reach 1.5 m), often tinged purple, ± clothed all over with simple, stout, often multicellular, white or hyaline, ± spreading, usually eglandular hairs 1–4 mm long, and also rigid, curved at the tip, appressed to spreading, often minute, hairs, furnished with ± sessile glands too

Pedicel

Pedicel 2–5(9) mm long, villous or puberulous, occasionally subglabrous, in fruit elongated to 9(10) mm

Ovary

Ovary 0.8–0.9 × 0.6–0.7 mm, ellipsoid or ± globose, glabrous.

Style

Style 2–3 mm long, filiform, ± curved upwards

Note

The specimens Torre et al. in Torre 17705, 18035 and 19042, from the Tete Province of Mozambique have relatively large leaves, with petioles up to 8 cm long and laminas up to 10.5 × 7.5 cm. The specimen Flanagan 3305, from Zimbabwe, referred to by Wild, loc. cit. (1953) as P. minima, but not seen by me, is probably this species. Chromosome number: 2n=24

Distribution

Mozambique Zambia Zimbabwe Malawi ZAM N, ZAM W, ZAM C, ZAM S, ZIM N, ZIM E, MAL C, MAL S, MOZ Z, MOZ T, MOZ MS Native to tropical America (Mexico and Antilles), now extending northwards to the United States and southwards throughout Central America and the Caribbean to Bolivia.

Habit

Erect to decumbent or prostrate, weak or occasionally ± robust, ± dichotomously much branched, annual herb, (0.05)0.1–1 m high (elsewhere said to reach 1.5 m), often tinged purple, ± clothed all over with simple, stout, often

multicellular, white or hyaline, ± spreading, usually eglandular hairs 1–4 mm long, and also rigid, curved at the tip, appressed to spreading, often minute, hairs, furnished with ± sessile glands too.

Branches

Branches angular or angular-ribbed, striate, drying ± sulcate, sparingly villous, with ± long hairs mainly on the emergent parts and also short and minute hairs somewhat localized near the nodes, occasionally subglabrous. Branches angular or angular-ribbed, striate, drying ± sulcate, sparingly villous, with ± long hairs mainly on the emergent parts and also short and minute hairs somewhat localized near the nodes, occasionally subglabrous

Leaves

Leaves solitary or geminate; petiole 0.5–4.5(8) cm long, often slightly winged, rather sheathing at the base; lamina membranous or ± fleshy, 1.5–7.5(10.5) × 0.8–4.5(7.5) cm, ovate to lanceolate, sometimes rhombic, ovate-oblong, elliptic or oblanceolate, base shortly cuneate or attenuate, sometimes obtuse, rounded, truncate or subcordate, and often oblique or unequal-sided, sometimes ± decurrent into the petiole, apex acuminate, acute or obtuse, entire to somewhat coarsely sinuate-dentate, rarely dentate, the teeth unequal, ± triangular, obtuse, the sinuses rounded, both surfaces ± sparsely pilose or subglabrous but pubescent along the nerves and near the margins; minor leaves with lamina 0.5–0.7 × 0.3–0.4 cm, elliptic, sometimes present. Leaves solitary or geminate; petiole 0.5–4.5(8) cm long, often slightly winged, rather sheathing at the base; lamina membranous or ± fleshy, 1. 5–7.5(10.5) × 0.8–4.5(7.5) cm, ovate to lanceolate, sometimes rhombic, ovate-oblong, elliptic or oblanceolate, base shortly cuneate or attenuate, sometimes obtuse, rounded, truncate or subcordate, and often oblique or unequal-sided, sometimes ±

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Flowers

Flowers solitary, inserted by the side of the petiole-base appearing axillary, erect to pendulous; pedicel 2–5(9) mm long, villous or puberulous, occasionally subglabrous, in fruit elongated to 9(10) mm. Flowers solitary, inserted by the side of the petiole-base appearing axillary, erect to pendulous.

Calyx

Calyx (1.5)2–3.5(4) mm long, 1.5–2.5(3) mm across at the base of the lobes, campanulate, sub-angled-ribbed, truncate or invaginated at the base, with scattered, stout, white or hyaline, ± spreading, long hairs, or puberulous especially along the ribs, on the inside glabrous except for the lobes with ± dense, minute indumentum towards the apex, near the margins and sometimes also along the midribs; lobes subequal, (0.3)0.5–1.2(1.5) × 0.7–1(1.2) mm, ± deltate or ovate-triangular to triangular-lanceolate, acute or obtuse, sometimes sub-acuminate, rarely sub-truncate; in fruit greenish, usually drying purple-veined, (10)12–20(23) × (8)10–15(20) mm, globose to ± ovoid, 10-angled-ribbed or 10-ribbed, slightly obtuse or apiculate at the summit, half to almost filled by the fruit, subglabrous to pilose, mostly with long hairs especially along the ribs and nerves, often the ribs with widely spaced small teeth or enations derived from the hair bases, the lobes (0.5)1–2.5(3) × 1–2 mm. Calyx (1. 5)2–3.5(4) mm long, 1. 5–2.5(3) mm across at the base of the lobes, campanulate, sub-angled-ribbed, truncate or invaginated at the base, with scattered,

stout, white or hyaline, ± spreading, long hairs, or puberulous especially along the ribs, on the inside glabrous except for the lobes with ± dense, minute indumentum towards the apex, near the margins and sometimes also along the midribs; lobes subequal, (0.3)0.5–1. 2(1. 5) × 0.7–1(1. 2) mm, ± deltate or ovate-triangular to triangular-lanceolate, acute or obtuse, sometimes sub-acuminate, rarely sub-truncate; in fruit greenish, usually drying purple-veined, (10)12–20(23) × (8)10–15(20) mm, globose to ± ovoid, 10-angled-ribbed or 10-ribbed, slightly obtuse or apiculate at the summit, half to almost filled by the fruit, subglabrous to pilose, mostly with long hairs especially along the ribs and nerves, often the ribs with widely spaced small teeth or enations derived from the hair bases, the lobes (0.5)1–2.5(3) × 1–2 mm

Corolla

Corolla greenish-yellow or yellow to white or greenish-cream, slightly purplish marked or apparently unmarked, more rarely blotched with 5 ± dark purple to brown markings not strongly contrasting with the surrounding limb, (3)4–5(7) mm long, tubular-campanulate, often narrowly so; tube glabrous, on the inside with a few hyaline hairs from the insertion of the stamens upwards; limb (2)3–6 mm across, shortly 5-lobed, erect or spreading, rarely reflexed when fully expanded, glabrous or puberulous on the parts not folded in bud, glabrous inside, ciliate. Corolla greenish-yellow or yellow to white or greenish-cream, slightly purplish marked or apparently unmarked, more rarely blotched with 5 ± dark purple to brown markings not strongly contrasting with the surrounding limb, (3)4–5(7) mm long, tubular-campanulate, often narrowly so; tube glabrous, on the inside with a few hyaline hairs from the insertion of the stamens upwards; limb (2)3–6 mm across, shortly 5-lobed, erect or spreading, rarely reflexed when fully expanded, glabrous or puberulous on the parts not folded in bud, glabrous inside, ciliate

Stamens

Stamens included or slightly exserted, equal or subequal; filaments (1)1.5–3(3.5) mm long, filiform, attached to the corolla tube near the base, furnished with few hairs; anthers yellowish, sometimes blue-margined, 0.8–1 mm long, ± oblong or elliptic in outline, straight after anthesis. Stamens included or slightly exserted, equal or subequal; filaments (1)1. 5–3(3.5) mm long, filiform, attached to the corolla tube near the base, furnished with few hairs; anthers yellowish, sometimes blue-margined, 0.8–1 mm long, ± oblong or elliptic in outline, straight after anthesis

Disc

Disk 0.2–0.3 mm high, fleshy, glabrous. Disk 0.2–0.3 mm high, fleshy, glabrous

Pistil

Ovary 0.8–0.9 × 0.6–0.7 mm, ellipsoid or ± globose, glabrous; style 2–3 mm long, filiform, ± curved upwards.

Fruits

Fruit greenish-yellow or pale yellow, occasionally yellow, subsessile or with a gynobase up to 1 mm long on the invaginated base of the erect to pendulous calyx, (5)6–10 mm in diameter, ± globose or slightly ovoid, viscid. Fruit greenish-yellow or pale yellow, occasionally yellow, subsessile or with a gynobase up to 1 mm long on the invaginated base of the erect to pendulous calyx, (5)6–10 mm in diameter, ± globose or slightly ovoid, viscid

Seeds

Seeds light brown or yellowish, (1.5)1.8–2 × (1.5)1.6–1.8(2) mm, ovate to orbicular in outline, sometimes reniform, reticulate-foveolate. Seeds light brown or yellowish, (1. 5)1. 8–2 × (1. 5)1. 6–1. 8(2) mm, ovate to orbicular in outline, sometimes reniform, reticulate-foveolate

Action and uses:

It has been hypothesized that physalis extract affords protection by impairing CCl4 mediated lipid peroxidation, through decreased production of free radical derivatives. Which methanolic extract of *Physalis minima* leaves was investigated on ethanol induced ulcer models and pylorus ligation in wistar rats. The present study indicates that *Physalis minima* leaves extract have potential anti-ulcer activity in both models. This results may further suggests that methanolic extract was found to possess anti-ulcerogenic as well as ulcer healing property, which might be anti-secretory activity. As Physalis in rich in Flavonoids it is having hepatoprotective effect studies suggest that the aqueous and ethanol extracts prepared from the whole plant of these species were evaluated for their antihepatoma activity. The results conclude that plant possesses potent antihepatoma activity and its effect on apoptosis is associated with mitochondrial dysfunction. *Physalis lagascae roots* extract has potent anti-asthmatic activity. Its anti-asthmatic property probably acts via a reduction in inflammatory mediator's release. Thus the *Physalis lagascae* has significant anti-asthmatic property.

5. MATERIALS AND METHODS

PLANT MATERIALS

The aerial parts of ***Physalis lagascae***was collected from Tirunelveli district in September 2017 and authenticated by **Dr.G.Johnsichristobel., Ph.D.** Head of the Department & Research center, Department of Botany, Marthandam-629 165, Kanyakumari District. A voucher specimen of *Physalislagascae*(JKKM/POC/CC-285) was deposited in the department of pharmaceutical chemistry in JKKMMRF'S - Annai Sampoorani Ammal College of Pharmacy, Komarapalayam for future reference. The air dried aerial parts of the plant material was dried at room temperature, pulverized by a mechanical grinder, sieved through 40 mesh and then stored in an air tight and light resistant container for further use.

PRELIMINARY PHYTOCHEMICAL STUDIES

PREPARATION OF EXTRACT

About 1 kg of coarsely powdered plant material was first extracted with ethanol for 72 hours. The extract was concentrated using rotary evaporator to get solid residue. The marc left was removed, dried .Then the solvent changed and again successively extracted with chloroform. Then the product was collected and the solvent again removed. Then it again extracted with the ethyl acetate. The product also collected and the solvent again changed with petroleum etheruntil complete extraction was effected for 72 hrs. The product collected separately subjected to distillation. The percentage yield of each extract was calculated on dry weight analysis

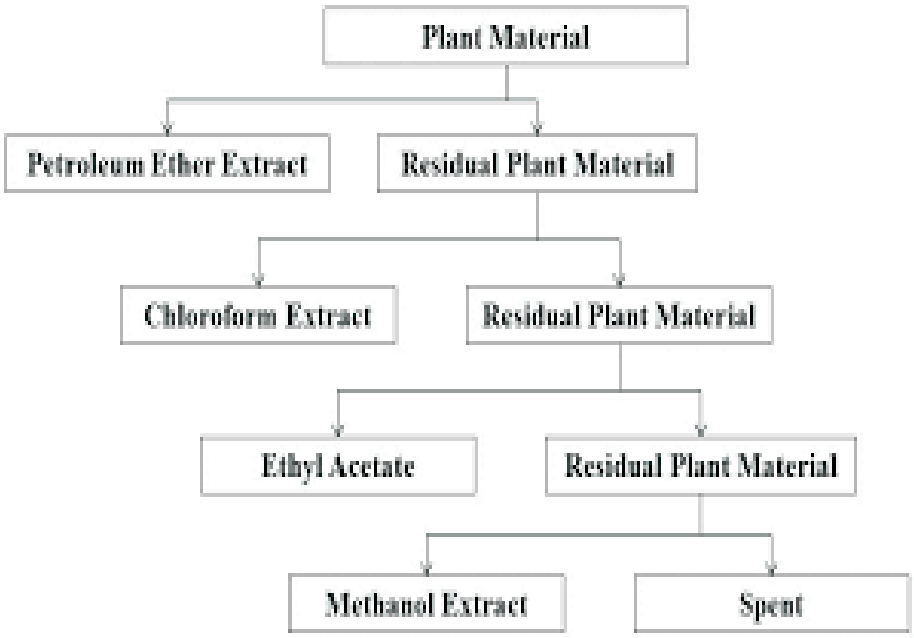


Fig. 8: Scheme of extraction of the aerial parts of *Physalis lagascae*

1. Detection of carbohydrates and glycosides

a) Molish's test

To small amount of sample and water solution add 2, 3 drops of alcoholic alpha naphthol solution and 2ml of concentrated sulphuric acid solution through the side s of the test tube. The formation of brown to violet ring indicate the presence of the carbohydrate**.**

b) Iodine test

To the filtrate iodine solution was added. The formation of deep to purple colour indicate the presence of the starch

c) Legal's Test

The filtrate was hydrolyzed with concentrated hydrochloric acid. To that add 1ml of pyridine and few drops of sodium nitro prusside. Finally make the solution

alkaline by using sodium hydroxide. The formation of pink to red color indicate the presence of glycosides.

d) Borntrager's Test

The filtrate was hydrolyzed with con.hydrochloric acid .it was added with chloroform and separates the chloroform layer and add equal volume of dilute ammonia solution. The ammonia layer acquired pink color indicate the presence of glycoside

2. Detection of alkaloids

Small quantity of extract was treated withdil.hydrochloric acid and filtered it. The collection of filtrate was used for the following tests

a) Mayer's Test

To the filtrate potassium mercuric iodide was added .Cream precipitate indicate the presence of alkaloids

b) Dragendroff's Test

To the filtrate potassium bismuth iodide was added .The formation of reddish brown precipitate indicate the presence of the alkaloids

c) Wagner's Test

To the filtrate iodine and potassium iodide was added, Reddish brown precipitate indicate the presence of the alkaloids

d) Hager's Test

The filtrate picric acid was added. The formation of yellow color precipitate indicate the presence of the alkaloids

3. Detection of phytosterols and steroids

Small quantity of the sample dissolved in chloroform and subjected to the following tests

a) Salkowsky Test

To the above solution 1ml chloroform and few drops of concentrated sulphuric acid was added. The formation of the red color indicate the presence of the steroids

b) Libermann Burchard Test

To the above solution 1ml of chloroform and few drops of con.sulphuric acid and 1ml of acetic anhydride. The formation of green color indicate the presence of steroid.

4. Detection of proteins and amino acids

Small quantity of extract was added with water and filter it.

a) Million's Test

To the above solution millions reagent was added. The formation of red color indicate the presence of proteins

b) Biuret Test

To the filtrate sodium hydroxide and copper sulphate solution added. Blue color indicate the presence of proteins, amino acid

4. Detection of tannins

a) To the 5ml of the extract 1ml of ferric chloride solution was added. The color change indicate the presence of tannins.

b) To the 5ml of the extract 1ml of aqueous potassium dichromate solution was added. The formation of blue color indicate the presence of tannins

5. **Detection of flavonoid**

a) To the extract sodium hydroxide was added. The formation of yellow color shows the presence of flavonoids

b) To the extract con.sulphuric acid was added. Color change shows the formation of the flavonoids

c) Shinodas Test: To the small quantity of sample dissolved in alcohol and add magnesiummetal and con.hydrochloric acid. Magenta color indicate the presence of flavonoids

d) Alkaline reagent test: To the extract sodium hydroxide solution was added it gives yellow color which shows the presence of flavonoids.

6. Detection of fixed oils

a) Spot Test: The small quantity of extract was pressed between two filter paper. The appearance of stain on the paper indicate presence of fixed oil

b)To the extract few drops of alcoholic potassium hydroxide and phenolphthalein were added and heated the solution. The color change indicate the presence of fixed oils

7. Detection of gums and mucilages

a) To the extract 25ml of alcohol was added with stirring and filtered it .Then precipitate was dried. The formation of swelling indicate the presence of gums and mucilages.

8. Detection of saponins

a) The extract was diluted with distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of form indicate the presence of saponins

9. Detection of steroids

a) Libermann Burchard Test

To the extract 1ml of the chloroform and con.sulphuric acid were added. Followedby acetic anhydride was added. Greenish color indicate the presence of steroid

Table 1: Data showing the preliminary phytochemical screening of the extracts of *Physalis lagascae*

Name of the Test	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Alcohol extract
Carbohydrate	-	+	+	+
Glycoside	-	-	-	-
Protein	+	-	+	-
Alkaloid	+	+	+	+
Saponins	-	-	-	+
Tannin	+	+	-	+
Phytosterols and steroids	+	+	-	+
Flavonoids	-	-	-	-
Fixed oil	+	+	+	+

6. CHROMATOGRAPHIC STUDIES

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

Thin layer chromatography studies are among the key identity tests in most pharmacopoeial monographs. Pharmacopoeial standards are typically used by industry as a basis for meeting QC requirements and current good manufacturing practices (cGMPs). An extension of TLC is high-performance thin layer chromatography (HPTLC) is robust, simplest, rapid, and efficient tool in quantitative analysis of compounds. HPTLC is an analytical technique based on TLC, but with enhancements intended to increase the resolution of the compounds to be separated and to allow quantitative analysis of the compounds. Some of the enhancements such as the use of higher quality TLC plates with finer particle sizes in the stationary phase which allow better resolution.

The separation can be further improved by repeated development of the plate, using a multiple development device. As a consequence, HPTLC offers better resolution and lower Limit of Detection (LODs). Visual detection is suitable for qualitative analysis, but a more specific detection method is needed for quantitative analysis and for obtaining structural information on separated compounds. UV, diode-array and fluorescence spectroscopy, mass spectrometry (MS), Fourier-transform infrared (FTIR), and Raman spectroscopy have all been applied for the in situ detection of analyte zones on a TLC plate.

High-performance thin-layer chromatography (HPTLC) is a form of thin-layer chromatography (TLC) that provides superior separation power using optimized coating material, novel procedures for mobile-phase feeding, layer conditioning, and improved sample application. It promotes for higher separation efficiencies, shorter analysis time, lower amounts of mobile phase, and efficient data

acquisition and processing. The major parameters that influence separation of the constituents within a mixture are the partition coefficients, retention factor (Rf), and capacity factor of the individual constituents on the plate, selectivity of the mobile and stationary phase to the solutes, and the plate height that decide the separation efficiency as well as resolution of the individual constituents within a mixture.

The partition coefficient is defined as the molar concentration of the analyte in the stationary phase to that in the mobile phase. Rf, a fundamental qualitative value, is expressed as the ratio of migration distances of an individual components of a mix relative to the mobile phase. Capacity factor (k) is a fundamental characteristic of a substance that determines its qualitative chromatographic behavior. It can be expressed as the ratio of the retention time of the substance in the stationary to that in the mobile phase and is influenced by the chemical nature of the two phases.

The separation number (SN) that influences separation power of HPLC is defined as the highest possible number of components that are completely separated in a mixture under a gradient-free isocratic TLC. The efficacy of separation of two components of a mixture in a chromatogram is termed as resolution and is influenced by the selectivity of the components between the stationary and the mobile phase, mobile phase flow rate influenced by particle size and solvent strength that influence capacity factors.

The usage of HPTLC is well appreciated and accepted all over the world. Many methods are being established to standardize the assay methods. HPTLC remains one step ahead when compared with other tools of chromatography. One of the available chromatographic techniques is HPTLC, which is used for the identification of constituents, identification and determination of impurities, and quantitative determination of active substances. The use of modern apparatus such

as video scanners, densitometers, and new chromatographic chambers, and more effective elution techniques, high-resolution sorbents with selected particle size or chemically modified surface, the possibility of combining with other instrumental methods, and development of computer programs for method optimization all make HPTLC an important alternative method to HPLC or gas chromatography.

Specifically, HPTLC is one of the ideal TLC technique for the analytical purposes because of its increased accuracy, reproducibility, and ability to document the results, compared with standard TLC. Because of this, HPTLC technologies are also the most appropriate TLC technique for conformity with GMPs.

HPTLC remains one of the most flexible, reliable, and cost-efficient separation technique ideally suited for the analysis of botanicals and herbal drugs. Used with standardized procedures, it guarantees reproducible results, a vital element in the routine identification of complex fingerprints of plant extracts and pharmaceutical products.It has established itself as the method of choice for handling complex analytical tasks involving herbal drugs and botanicals.

It has established itself as the method of choice for handling complex analytical tasks involving herbal drugs and botanicals. The unique combination of state-of-art instrumentation, standardized procedures, and solid theoretical foundations enables it to deliver reliable, cGMP-compliant results time after time. High-throughput analysis using HPLTC is being aimed at the rapid analysis of large numbers of compounds. This field has been expedited by the requirement to provide analytical support for multiple drug targets emerging from the field of molecular biology, human genetics, and functional genomics

HPTLC is one of the most widely applied methods for the analysis in pharmaceutical industries, clinical chemistry, forensic chemistry, biochemistry,

cosmetology, food and drug analysis, environmental analysis, and other areas. It is due to its numerous advantages, for example, it is the only chromatographic method offering the option of presenting the results as an image. Other advantages include simplicity, low costs, and parallel analysis of samples, high sample capacity, rapidly obtained results, and possibility of multiple detection.

The HPTLC technique is rapid, comparatively simple, robust, and extremely versatile. HPTLC not only confirm but also establish its identity. It is also an ideal screening tool for adulterations and is highly suitable for evaluation and monitoring of cultivation, harvesting, and extraction processes and testing of stability.

In recent years, HPTLC is a globally accepted practical solution to characterize small molecules in quality assessment throughout the developing world. HPTLC is used for purity control of chemicals, pesticides, steroids, and water analysis. HPTLC is also widely used for analysis of vitamins, water-soluble food dyes, pesticides in fruits, vegetables, and other food stuffs.Beate et al reported the analysis of stem cell lipids by offline HPTLC-MALDI-TOF MS. HPTLC is useful in detecting chemicals of forensic concern, including abuse drugs, poisons, adulterations, chemical weapons, and illicit drugs.

Instrumentation

Injection of the sample: Septum injectors are available; using which sample solution is injected. Sample can be injected when the mobile phase is flowing or it is stopped. A new advanced rotary valve and loop injector can be used to produce reproducible results. The detector: There are several ways of detecting when a substance has passed through the column. Generally UV spectroscopy is attached, which detect the specific compounds. Many organic compounds absorb UV light of various wavelengths. The amount of light absorbed will depend on the amount of a

particular compound that is passing through the beam at the time. Interpreting the output from the detector: The output is recorded as a series of peaks, each one representing a compound in the mixture passing through the detector and absorbing UV light. The area under the peak is proportional to the amount of substance, which is passed through detector, and this area can be calculated automatically by the computer linked to the display.

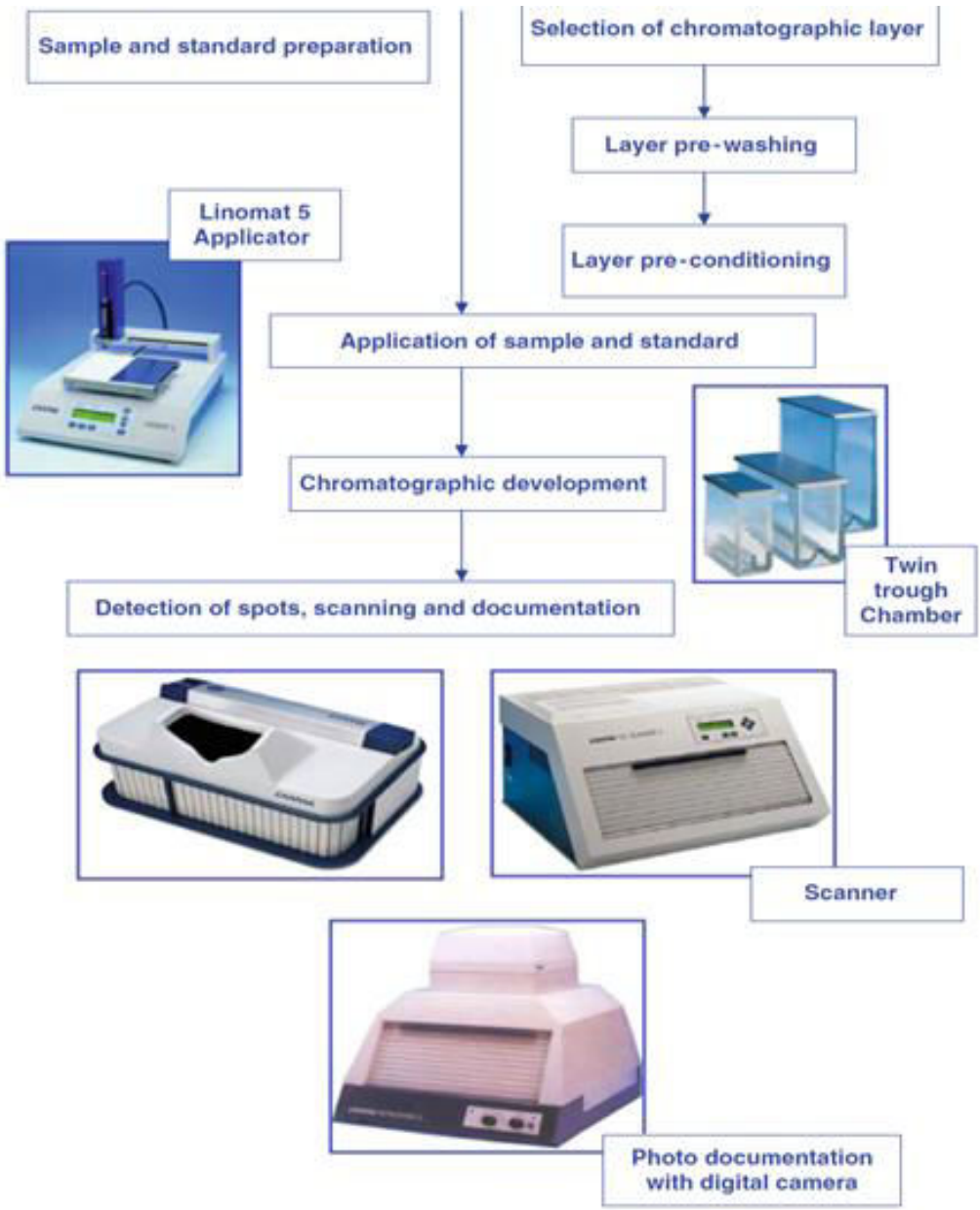


Fig. 9: Instrumentation

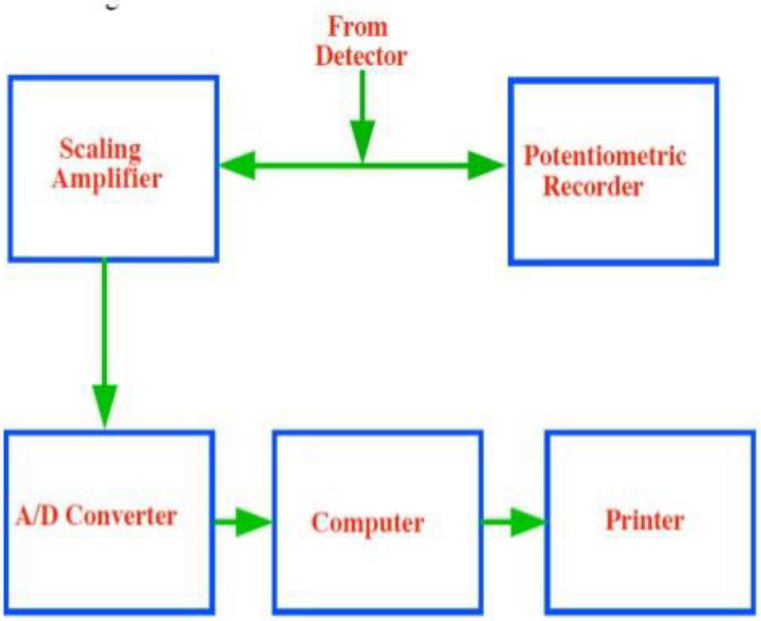


Fig. 10: HPTLC instrumentation

HPTLC ANALYSIS

ETHANOL EXTRACT OF *PHYSALIS LAGASCAE* WITH GALLIC ACID AND QUERCETIN STANDARD

Sample given

Sample **PL**	–	Sample PL Ethanolic extract
SG	-	Gallic acid standard as a reference marker
SQ	-	Quercetin standard as a reference marker

Procedure

Test solution preparation

The given plant sample was centrifuged at 3000rpm for 5min. This solution was used as test solution for HPTLC analysis.

Sample application

1µl of test solution and 2 µl of standard solutions were loaded as 5mm band length in the 3 x 10 Silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

Spot development

The sample loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase up to 90mm.

Photo-documentation

The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at visible light, UV 254nm and UV366nm.

Derivatization

The developed plate was sprayed with respective spray reagent and dried at 100°C in Hot air oven. The plate was photo-documented in Visible light mode using Photo-documentation (CAMAG REPROSTAR 3) chamber.

Scanning

Before derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 366nm. The Peak table, Peak display and Peak densitogram were noted. The software used was win CATS 1.3.4 version.

ANALYSIS DETAILS

Mobile phase

100% Chloroform

Spray reagent

Anisaldehyde sulphuric acid reagent.

Detection

Yellowish orange coloured zone at Visible mode/ Blue color fluorescent zone in UV 366nm mode was present in thetrack, it was observed from the chromatogram after derivatization, which maybe the **Presence of Quercetin** in the given standard.

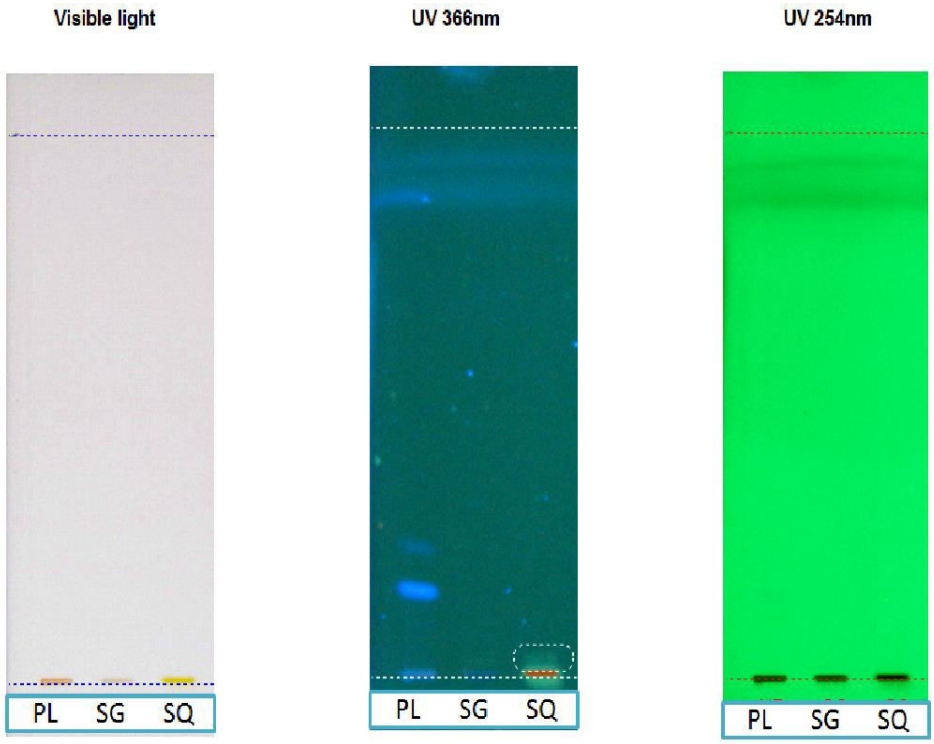
Gallic acid standard was not moved from the loading point for the given mobile phase.

Peak table

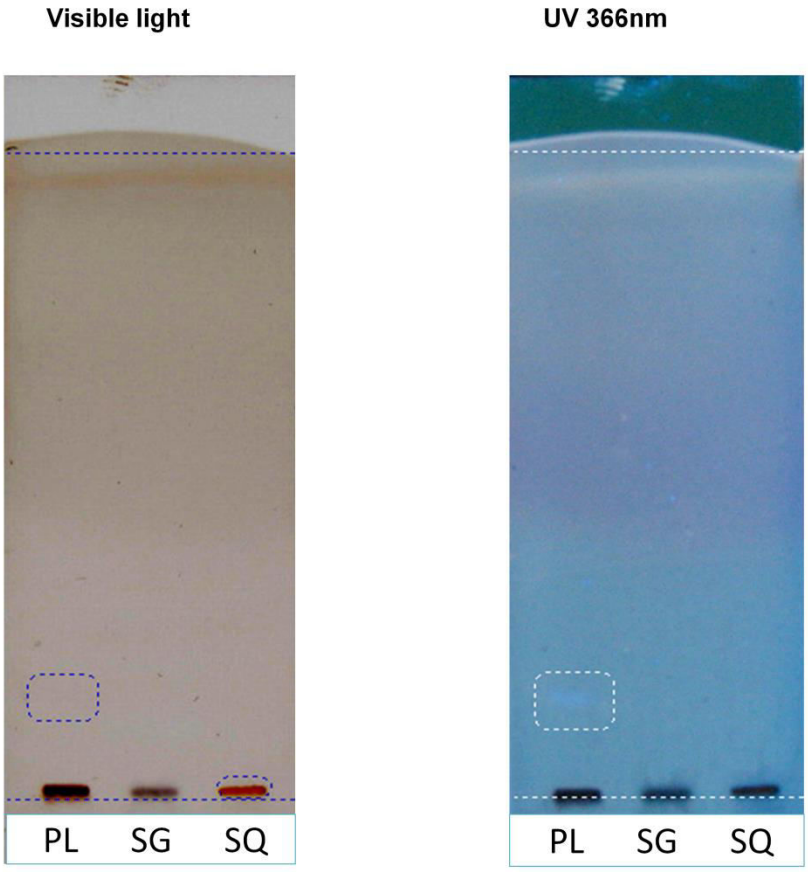
Track	Peak	Rf	Height	Area	Assigned substance
Sample PL	1	0.14	84.1	1356.3	Unknown
Sample PL	2	0.88	32.1	914.3	Unknown
Sample PL	3	0.92	45.6	1369.3	Unknown
SQ	1	16.6	22.93	147.4	Quercetin standard

Chromatogram

Before derivatization

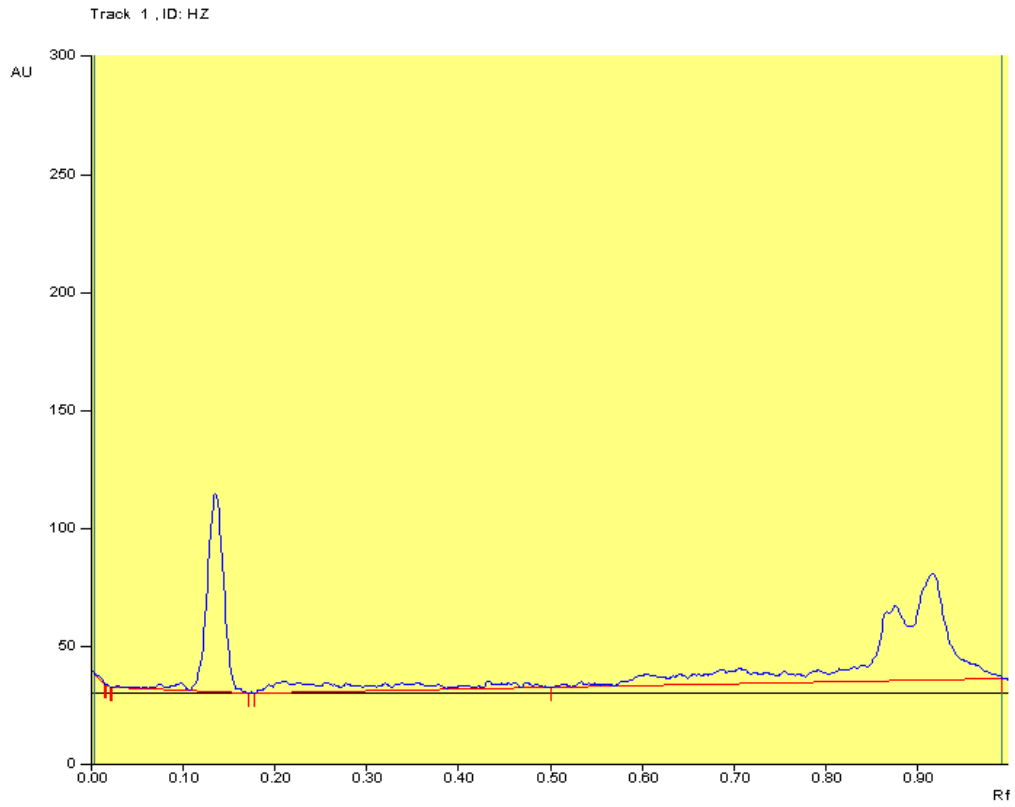


After derivatization



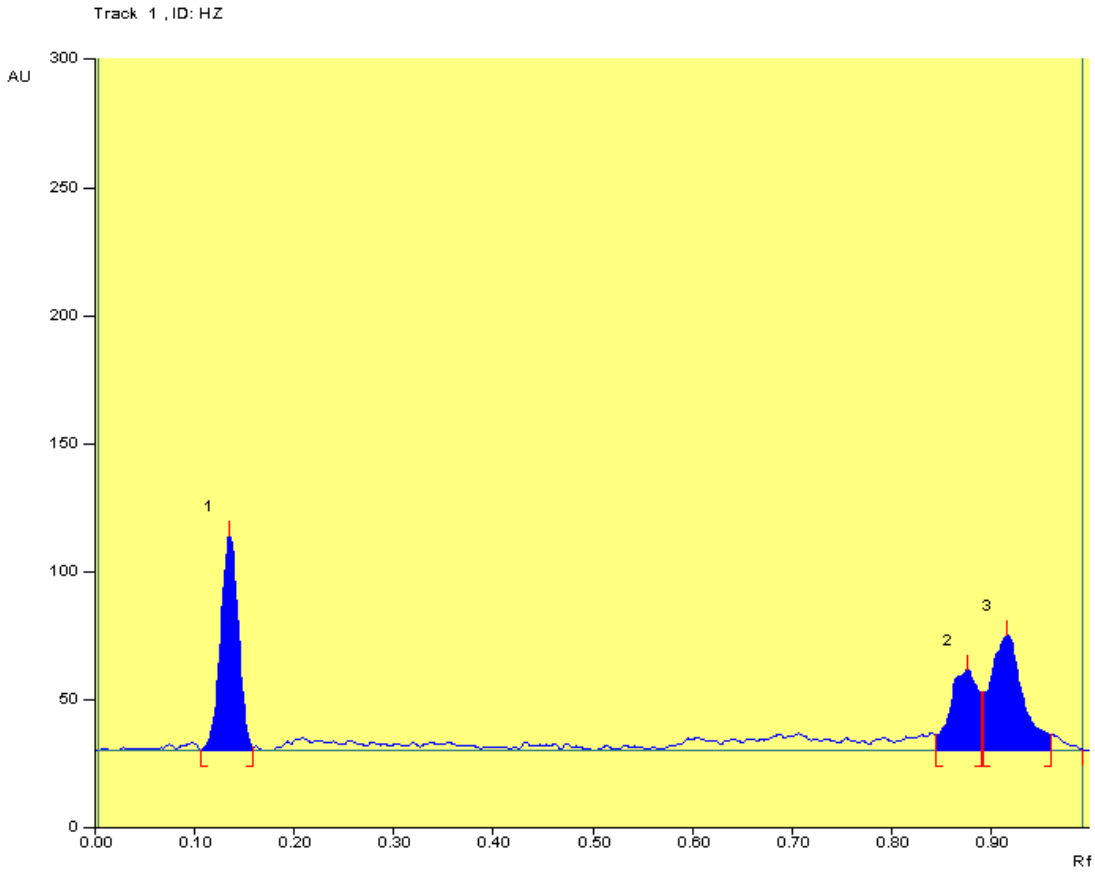
Track 1 – Sample PL plant extract sample- Baseline display

(Scanned at 366 nm)



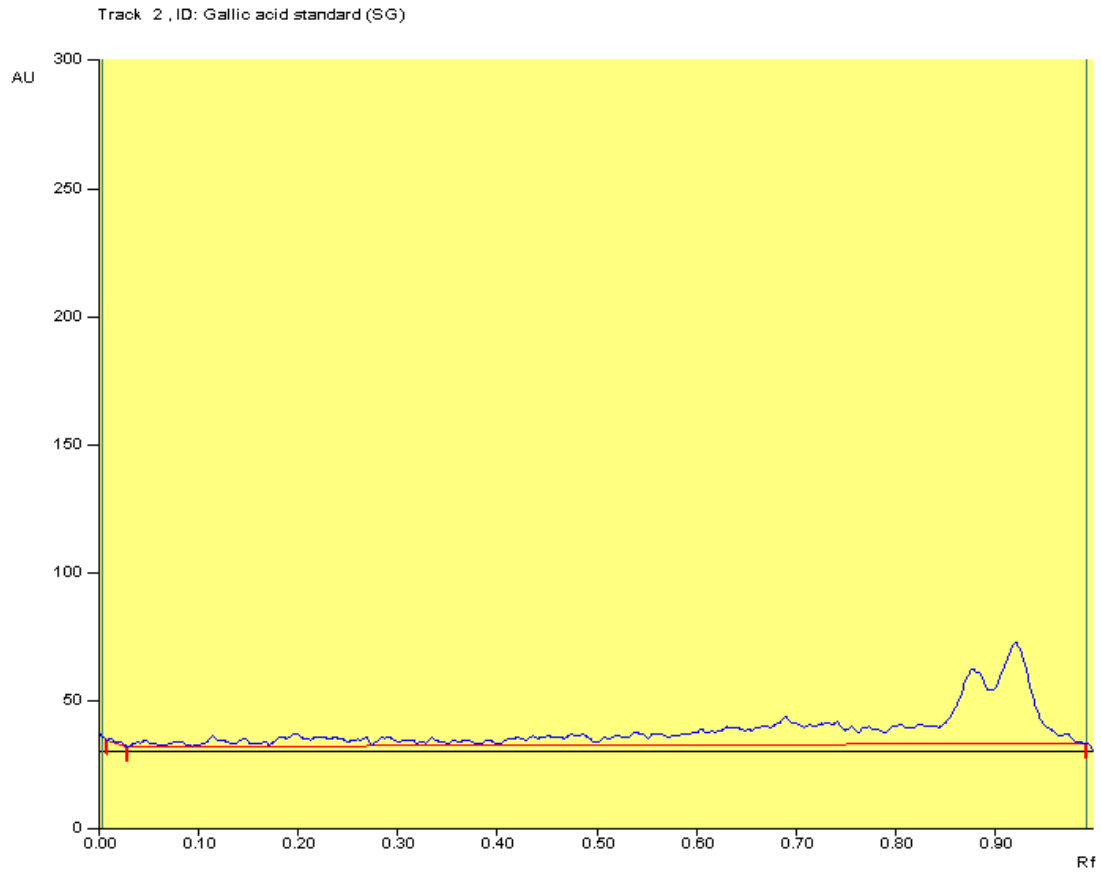
Track 1 – Sample PL plant extract sample - Peak densitogram display

(Scanned at 366 nm)



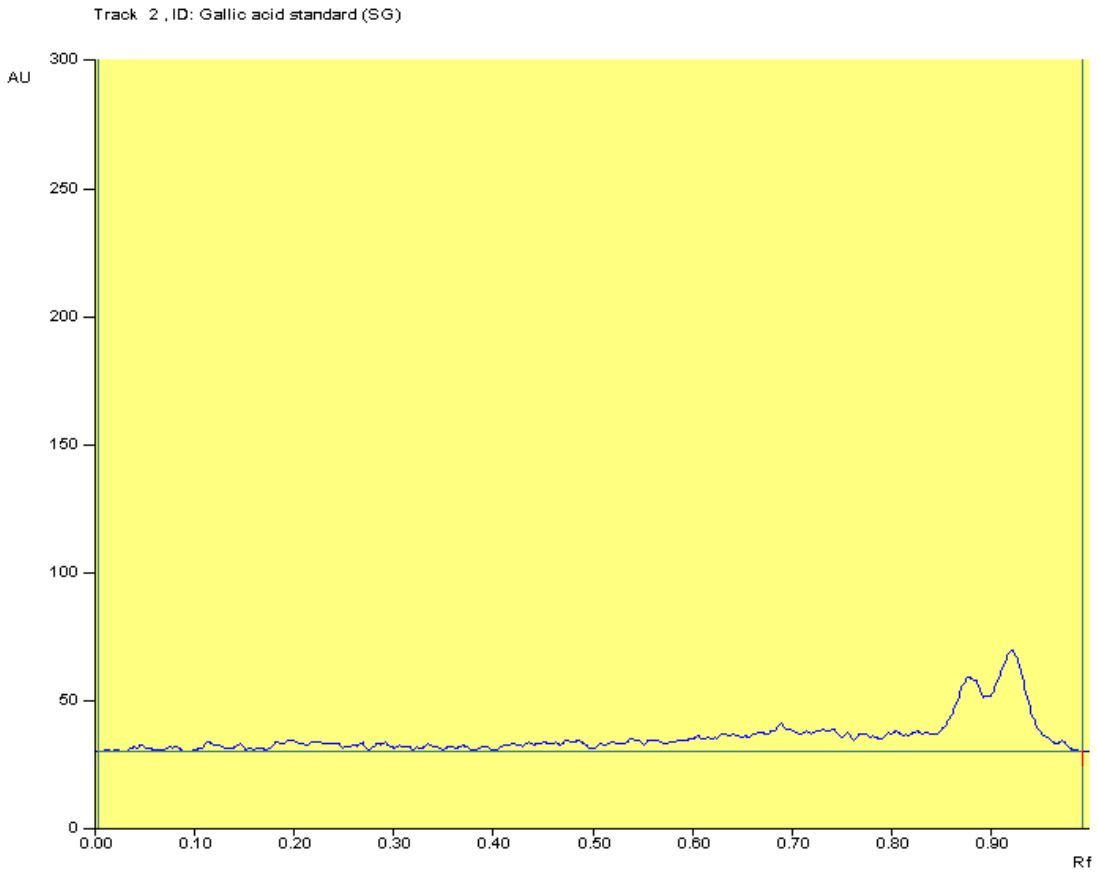
Track STD –Gallic acid standard Baseline display

(Scanned at 366 nm)



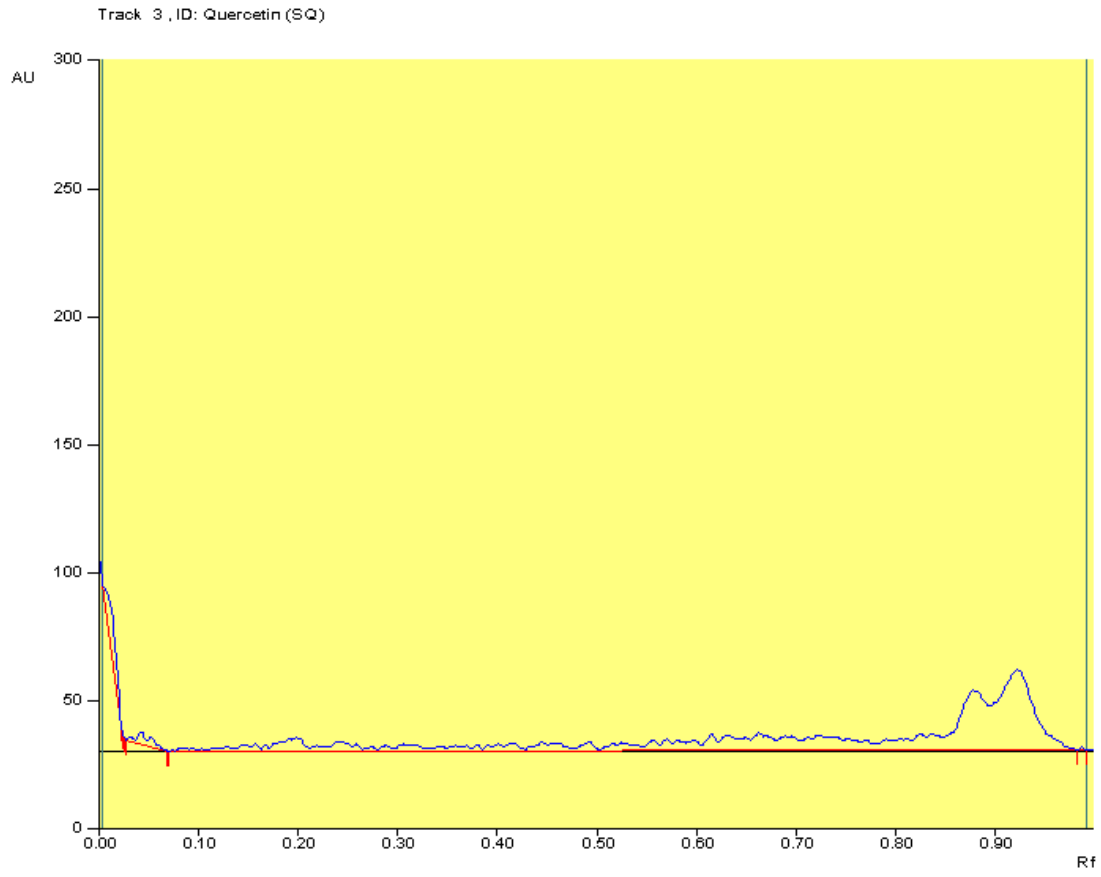
Track STD – Gallic acid standard Peak densitogram display

(Scanned at 366nm)



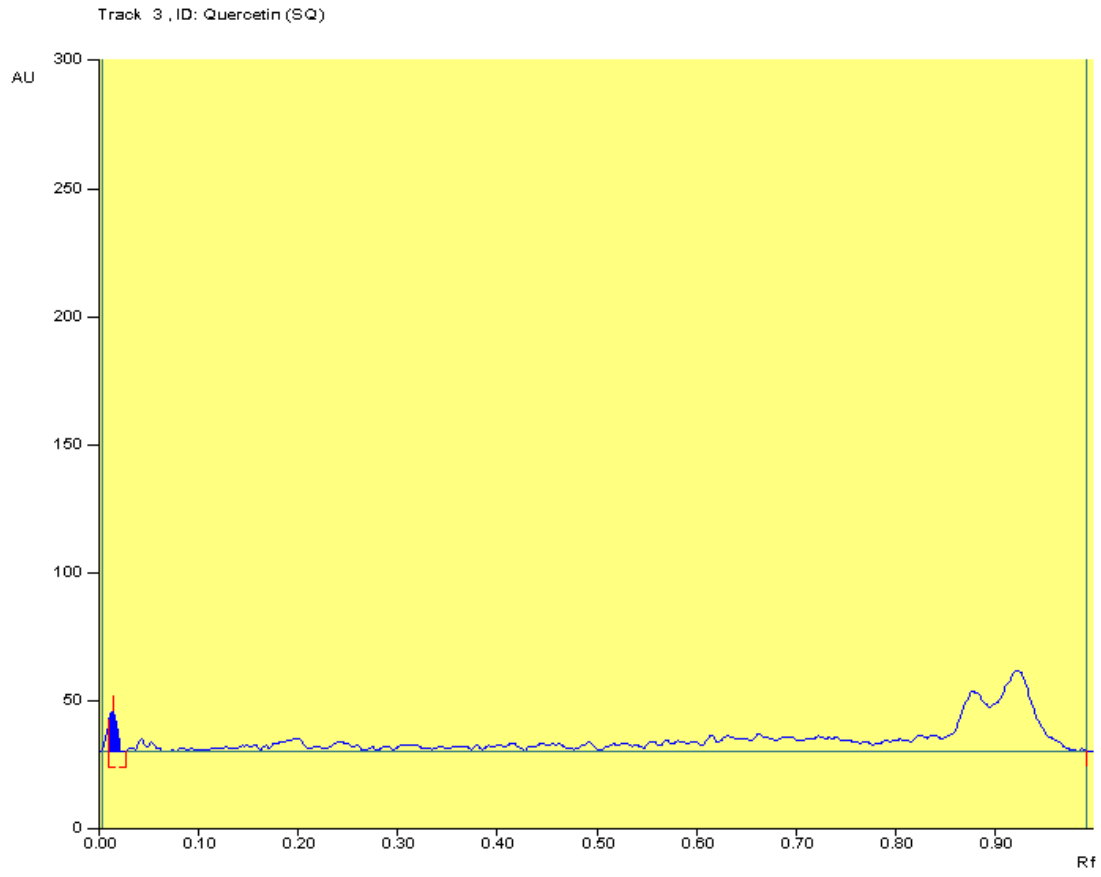
Track STD –Quercetin standard Baseline display

(Scanned at 366 nm)

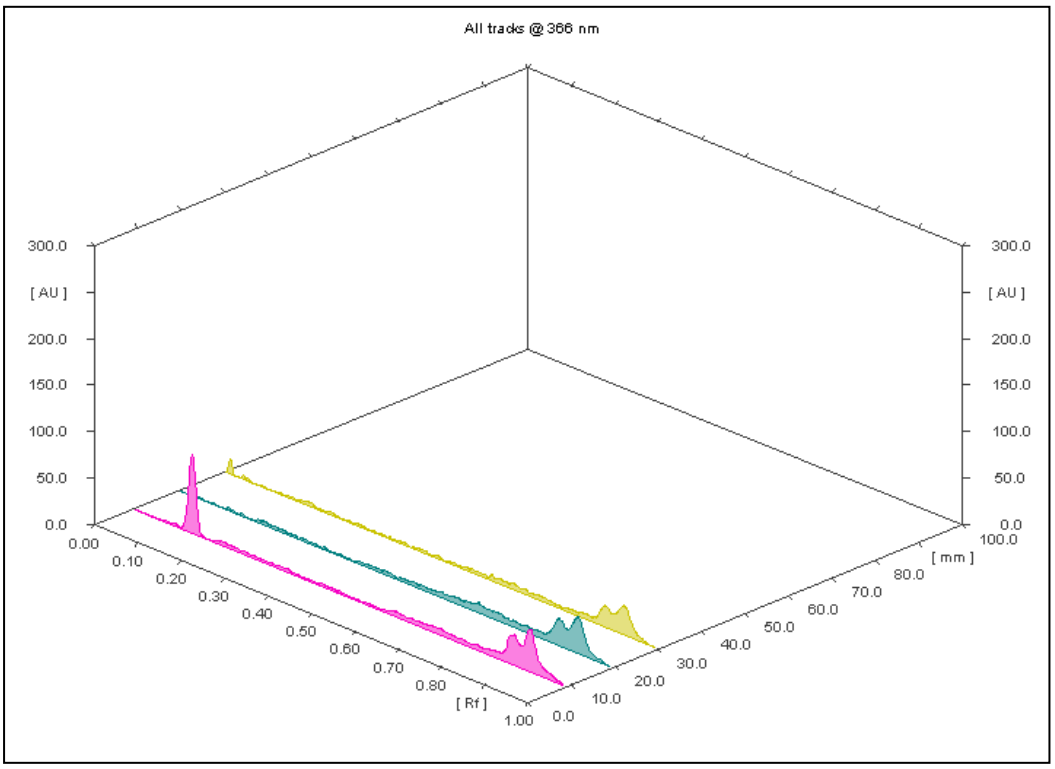


Track STD – Quercetin standard Peak densitogram display

(Scanned at 366nm)



3D display of all Tracks



NOTE

Sample **PL** – Sample PL Ethanolic extract

SG - Gallic acid standard as a reference marker

SQ - Quercetin standard as a reference marker

Here we used the Ethanolic extract of *Physalis lagascae*for the HPTLC. The garlic acid standard and Quercetine standard as a reference marker. The movement of the sample was comparedwith the other two components and finally decide the presence of compound

Yellowish orange coloured zone at Visible mode/ Blue color fluorescent zone in UV 366nm mode was present in thetrack, it was observed from the chromatogram after derivatization, which maybe the **Presence of Quercetin** in the given standard.

Gallic acid standard was not moved from the loading point for the given mobile phase.

Quercetin, a plant flavonol from the flavonoid group of polyphenols, is found in many fruits, vegetables, leaves, and grains; red onions and kale are common foods containing appreciable content of quercetin.It has a bitter flavor and is used as an ingredient in dietary supplements, beverages, and foods. Quercetin is a flavonoid widely distributed in nature.[2] The name has been used since 1857, and is derived from *quercetum* (oak forest), after *Quercus*. It is a naturally occurring polar auxin transport inhibitor. In plants, phenylalanine is converted to 4-coumaroyl-CoA in a series of steps known as the general phenylpropanoid pathway using phenylalanine ammonia-lyase, cinnamate-4-hydroxylase, and 4-coumaroyl-CoA-ligase. One molecule of 4-coumaroyl-CoA is added to three molecules of malonyl-CoA to form tetrahydroxychalcone using 7, 2′-dihydroxy-4′-

methoxyisoflavanol synthase. Tetrahydroxychalcone is then converted into naringenin using chalcone isomerase.

Naringenin is converted into eriodictyol using flavonoid 3′-hydroxylase. Eriodictyol is then converted into dihydroquercetin with flavanone 3-hydroxylase, which is then converted into quercetin using flavonol synthase.

Quercetin is the aglycone for of a number of other flavonoid glycosides, such as rutin and quercitrin, found in citrus fruit, buckwheat and onions. Quercetin forms the glycosidesquercitrin and rutin together with rhamnoseand rutinose, respectively. Likewise guaijaverinis the 3-*O*-arabinoside, hyperoside is the 3-*O*-galactoside, isoquercitin is the 3-*O*-glucosideand spiraeoside is the 4′-*O*-glucoside. CTN-986 is a quercetin derivative found in cottonseeds and cottonseed oil. Miquelianin is the quercetin 3-*O*-β-D-glucuronopyranoside.

In vitro pharmacology

Quercetin has been reported to inhibit the oxidation of other molecules and hence is classified as an antioxidant.Quercetin contains a polyphenolic chemical substructure that stops oxidation by acting as a scavenger of free radicals that are responsible for oxidative chain reactions Quercetin also activates or inhibits the activities of a number of proteins. For example, quercetin is a non-specific protein kinase enzyme inhibitor. Quercetin has also been reported to have estrogenic (female sex hormone-like) activities by activating estrogen receptors. Quercetin activates both estrogen receptor alpha (ERα) and beta (ERβ) with binding IC_{50} values of 1015 nM and 113 nM, respectively. Hence quercetin is somewhat ERβ selective (9 fold) and is roughly two to three orders of magnitude less potent than the endogenous estrogenic hormone 17β-estradiol.In human breast cancer cell lines, quercetin has also been found to act as an agonist of the G protein-coupled estrogen receptor (GPER).[39]

7. PHARMACOLOGICAL SCREENING

Invitro Anticancer Mechanism of Plant Compounds

Carcinogenesis is due to the presence of toxic chemicals, excessive use of alcohol, exposure to excessive sunlight and environmental toxins, some poisonous mushrooms, inherent genetic problems, ionizing radiation and several viruses. Angiogenesis, invasiveness and metastasis are the regular expression of progressive phase of the malignant disease. Treatment of cancer in developed countries is given by the combination of surgery, chemotherapy and radiation. This sort of treatment destroys the protective mechanism of cancerous cells and its metastasis but their toxic effect kills normal cells along with cancer cells. It decreases the hematological level and immune disorder making the patient liable to infections. Cytotoxicity screening test is the preliminary method for assortment of active plant extracts against cancer. Therapeutic value of medicinal plants is chiefly because of the presence of alkaloids and polyphenols, thus they are much concentrated than other secondary metabolites available in the plants. The cytotoxicity and genotoxicity of medicinal plants in terms of herbal extracts, infusions, essential oils and fractions is evaluated using cytogenic assays for their mutagenic and carcinogenic effects. The search for safety tools for the treatment of malignancy resulted in identifying plants and its compounds with properties like herbal adaptogen, anti-stress and immunomodulators. However many people still use traditional medicine as an alternative treatment for cancer. [45]

Current scenario of degenerative disease:

In spite of modern developments in prevention, diagnosis and therapy, cancer stays as a vast threat to human health and affects millions of people worldwide. In developing countries the cervical cancer (gynecological malignant

tumour) commonly causes harm to women health. Although the chemical components and mechanism of action of natural plants with anti-cervical cancer potential have been discussed, many others remain unknown. At present, breast cancer is the crucial cause of cancer-related death in women worldwide, although radiation and chemotherapy are accessible, they enclose loads of lethal and long-term side effects and even some of them are stable which can cause infertility. Malignant and aggressive ER (estrogen receptor) negative breast cancer is resistant to hormonal therapy.

Myelo-suppression and anemia are the two major problems encountered in chemotherapy as the result of reduction in erythrocytes or hemoglobin which has occurred due to iron deficiency/ hemolytic/ myelopathic conditions. Hematological toxicity of drugs triggers higher possibility of neutropenia with thrombocytopenia. Liver functions are commonly impaired among cancer patients who lead to the loss of functional integrity of the cell membrane resulting in elevated levels of aspartate and alanine amino transferase, alkaline phosphatase and serum bilirubin. Chemo-preventive agents of plant's origin react by producing nitrosoamines which inhibit carcinogen formation, as blocking agents that avert carcinogens from reacting or reaching to target sites and thus act as suppressing or anti-proliferative agents. Arginine functions as an essential substrate for the anti-tumour immune system especially T-lymphocytes. Nitric oxide (NO) may prevent malignant outgrow by activating tumour suppressor genes and causing apoptosis91. However, malignancies revert these anti-tumour mechanisms by interfering with arginine/NO metabolism by inducing myeloid derived suppressor cells (MDSC) that produce the arginine converting enzymes arginase and inducible nitric oxide synthase (iNOS). This may contribute to malignant outgrow and development of cancer associated opportunistic syndromes (cancer cachexia). Now, the major criteria for treatment are metabolic failure in cancer patients and multidrug resistance- associated protein

(MRP1/ABCC1). Accordingly the curiosity is rising in the identification of naturally occurring molecules with chemo-preventive and chemotherapeutic properties. [23]

Carcinogenesis and Chemoprevention:

Free radicals are generated in living systems as part of metabolic activities and their levels are regulated by cellular antioxidant system. Uncontrolled levels of reactive oxygen species (ROS) can cause cell membrane damage and DNA mutation which further results in development of many diseases such as cancer, brain disorders and cardiovascular diseases. Free radicals react with purines, pyrimidines and chromatin protein (cellular biomolecules) leading to base modifications, unstable genomes and genetic alterations. ROS generation alters the activity of many early response genes and transcription factors66. Plant polyphenols act on multiple cancer-inflammation and reactive oxygen/reactive nitrogen species (ROS/RNS)-mediated pathways that inhibit oxidative stress and DNA damage that is implicated in mutagenesis, carcinogenesis and premature ageing. Oxidative stress can be premodulated with the interference of exogenous antioxidants like curcumin, resveratrol, catechin and gensitein. Consumption of diet containing high portion of vitamins has confirmed advantageous in cancer prevention.

Occurrence of Secondary cancer:

High morbidity and mortality rates among the cancer patients are due to the presence of advanced stages of cancer cells having highly invasive potential. Inhibition of invasion and metastasis may be and invasiveness to cancer cells during tumour distribution. Matrix metalloproteinases (MMPs), a family of Zn dependent endopeptidases have a central functional role in tumour cell migration, spreading, invasion and metastasis. Reversion- inducing cysteine- rich protein with kazal

motifs (RECK) acts as a negative regulator of MMPs a good approach to cancer treatment. Epithelial mesenchymal transition (EMT) provides greater motility [33]

Invasion:

Solanum nigrum Linn. Consists of steroidal glycoalkaloid, α-solanine which increases the expression of epithelial marker Ecadherin and decreases the expression of mesenchymal marker vimentin and therefore it suppresses epithelial mesenchymal transition (EMT). The glycoalkaloid, α-solanine reduces the mRNA level of matrix metalloproteinase2 (MMP2), MMP9 and extracellular matrix metalloproteinase inducer (EMMPRIN) but increases the expression of RECK and TIMP-1 and 2 (tissue inhibitors of metalloproteinases). It also suppresses the phosphorylation of phosphatidylinositide 3 kinase (PI3K), Akt (protein kinase B) and ERK (extracellular signal-regulated kinases). Thus α-solanine down regulates oncogenic microRNA21 (miR-21) and upregulates tumour suppressor miR138 expression to suppress the invasion of prostate cancer cell.

Metastasis:

Cancer occurs when the cells gets damaged at nucleic acid level in a progressive way resulting in cancer stem cells that possess a malignant phenotype. Malignant tumour cells are considered to have the capacity to metastasize. Early breast cancer becomes metastatic after many years of its occurrence. Metastasis is the foremost reason of mortality among cancer patients. When the tumour cells have come to respite in another site, they enter the vessel walls, continue to multiply and ultimately form another tumour. MMPs are significant in tumourigenesis and metastasis. They cleave the extracellular matrix (ECM) substrates. Degradation of ECM is a key event in tumour progression, invasion and metastasis26. MMP-2 and -9 are important molecules for cancer invasion highly expressed in bladder and colon

cancer cells.23, 156 TIMPs are naturally occurring inhibitors of MMPs which prevent catalytic activity by binding to activated MMP62. Spontaneous and experimental metastasis to the liver is increased in mice expressing antisense TIMP-176. [43]

Indigenous treatment:

More than 60% of cancer patients use vitamins or herbs as therapy65. In traditional folk medicine, *Curcuma longa* L. (turmeric) is used for centuries as cancer treating medicine. Curcumin (or diferuloylmethane) suppress mutagenesis, inhibits nuclear factor- kB (NF-kB) activation, suppression of cyclin D1, induction of cytochrome C release, activation of caspases and also has anti-angiogenic effects through the down regulation of VEGF (Vascular Endothelial Growth Factor) 1. Allicin, a compound isolated from *Allium sativum* L. inhibits the activation of procarcinogens. *Murraya koenigii* L. leaves rich in polyphenols inhibit the proteolytic activity of the cancer cell proteasome and cause cell death122. It shows strong cytotoxicity against oral cavity cancer (KB), breast cancer (MCF7) and small cell lung cancer (NCI-H187) human cell lines. Series of medicinal plants were used specifically and constantly in traditional medicine is because of their spectrum of pharmacological activities related with the biologically active chemicals. [20]

Traditional plants:

A global broad-spectrum thought of medicinal plant users are ―the drugs derived from plants are always safe because they are natural‖. Plants are generally used in Ayurvedic medicinal practices to promote self-healing, good health and longevity. However, numerous scientific evidences had revealed that many plants are included as food or drug in traditional medicine.. Mostly previous works were focused on plant compounds like curcumin, β - carotene, stigmasterol, lupeol,

ursolic acid and β - sitosterol. Over exploitation of particular group of plants will cause problems in diversity and possible extinction. Other plants are preferable, as they may have novel potent compounds with therapeutic targets such as anti-mutagenic, anti-carcinogenic, anti- angiogenesis, anti- invasive, anti- metastasis, anti- proliferation, anti- inflammatory and apoptotic activity.

The International Agency for Research on Cancer estimates of the incidence of mortality and prevalence from major types of cancer, at national level, for 184 countries of the world revealed that there were 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide [1]. By 2030, it is projected that there will be 26 million new cancer cases and 17 million cancer deaths per year.

Today, despite considerable efforts, cancer still remains an aggressive killer worldwide. Moreover, during the last decade, novel synthetic chemotherapeutic agents currently in use clinically have not succeeded in fulfilling expectations despite the considerable cost of their development.

Therefore there is a constant demand to develop new, effective, and affordable anticancer drugs. From the dawn of ancient medicine, chemical compounds derived from plants have been used to treat human diseases. Natural products have received increasing attention over the past 30 years for their potential as novel cancer preventive and therapeutic agents. In parallel, there is increasing evidence for the potential of plant-derived compounds as inhibitors of various stages of tumorigenesis and associated inflammatory processes, underlining the importance of these products in cancer prevention and therapy. [(49)]

Approximately 60% of drugs currently used for cancer treatment have been isolated from natural products and the plant kingdom has been the most significant

source. These include vinca alkaloids, Taxus terpenes, Camptotheca alkaloids, and Podophyllum lignans. Currently, of 16 new plant-derived compounds being tested in clinical trials, 13 are in phase I or II and three are in phase III. Among these compounds, flavopiridol, isolated from the Indian treeDysoxylum binectariferum, and meisoindigo, isolated from the Chinese plant Indigofera tinctoria, have been shown to exhibit anticancer effects with lesser toxicity than conventional drugs. Medicinal plants constitute a common alternative for cancer treatment in many countries around the world. At this time, more than 3000 plants worldwide have been reported to have anticancer properties. Globally, the incidence of plant-derived products for cancer treatment is from 10% to 40% with this rate reaching 50% in Asiatic patients In Europe alone expenditure for anticancer herbal products is estimated to be 5 billion dollars per year

Israel, with its diverse climatic and geographic conditions, is home to some 2400 plant species Situated in a transition zone between Mediterranean woodlands in the north, shrubby formations and herbaceous vegetation in the east and south, shrub-steppes and extreme desert areas in the southern Negev, and tropical vegetation in the hottest parts of the country, Israel possesses a great diversity of species many of which are endemic only to this area. With a long history of traditional use spanning many centuries, the medicinal plants of Israel present a unique opportunity for focused screening based on their ethnobotanical use. [30]

In the current study we initially examined the effects of 17 whole plant extracts (ethanol extraction) of Israeli plants on human tumor cell lines as well as human primary cancer cultures. The three most effective plant extracts were then selected for additional research focusing also on the nature of cell death caused by these plant extracts. Our hypothesis was that whole cell extracts might contain

multiple molecules with antitumor activities and be very effective in killing human cancer cells.

The 3 plant extracts investigated were Urtica membranacea (Urticaceae) (referred to as extract number 5 in the study), Artemesia monosperma (Asteraceae) (referred to as extract number 10), and Origanum dayi Post (Labiatae).

All plants were investigated as part of the Middle Eastern Medicinal Plant Project (MEMP), an initiative of The Natural Medicine Research Centre (NMRC) dedicated to the ethnobotanical investigation, domestication, conservation, and reintroduction and focused screening of Israeli medicinal flora

All three selected plant extracts exhibited dose- and time-dependent killing capabilities in various human derived haematological and solid tumor cell lines and in primary cultures established from patients' biopsies. The killing activity was specific toward tumor cells, as the extracts had no effect on primary cultures of healthy human cells (lymphocytes and fibroblasts). Several experiments were carried out to characterize the plant extracts' activities. Using various methods it was found that, using whole plant extracts, cell death was caused via apoptosis.[48]

8. INVITRO ANTI-CANCER ACTIVITY

MTT Assay

Quantification of cell viability and proliferation form the fundamental for numerous in vitro assays in response to external factors. An MTT assay is a colorimetric assay based on assessing the cell metabolic activity. A549 Lung adenocarcinoma cell line was used to see the cytotoxic potential of a new drug for initial screening of apoptosis or necrosis. The biochemical mechanism behind the MTT assay involves NAD(P)H-dependent cellular oxidoreductase enzyme that converts the yellow tetrazolium MTT [3-(4, 5- dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] into insoluble (E,Z)-5-(4,5-dimethylthiazol-2-yl)- 1,3-diphenylformazan (formazan). The formed formazan can be dissolved with dimethyl sulfoxide (DMSO) to give a purple color with characteristic absorption at 540 nm. Intensity of purple color is directly proportional to the cell number and thus indicating the cell viability.

When a new drug, either natural source or synthesize, is under investigation needs to examine its safety to the host cell or the cytotoxic effect in cancer cell. This is well-known as the cell viability test. This viability cell test may vary from the simple one to the completed one. For example, exposure of cell to trypan blue can be useful to identify the viable cell (unstained) using a microscope. Dead cell is not stained with trypan blue (Strober, 2001).

This method is based on the cell membrane permeability. However, trypan blue staining cannot be used to distinguish between the healthy cells and the cells that are alive but losing cell functions. Another common method of examining the cell viability is the estimation of lactate dehydrogenase (LDH) level. LDH is present within the cytoplasm. When the integrity of a cell membrane is lost, then the

cytoplasmic LDH comes out of the cell, the LDH concentration in the extracellular medium is increased. This method is less sensitive than the MTT assay (Fotakis and Timbrell, 2006). Other methods used for the assessment of cell viability based on various cellular functions such as enzyme activity, cell membrane permeability, cell adherence, ATP production, co-enzyme production, and nucleotide uptake activity.

Among them tetrazolium (MTT) is one of the most frequently methods. This method uses colorimeter to determine the cell viability (Mosmann et al., 1983). The MTT reagent yields low background absorbance values in the absence of cells. In MTT assay, the linear relationship between metabolically active cells and the color produced is established, thus allowing an accurate quantification of changes in the rate of cell death or proliferation (van de Loosdrecht et al., 1994). MTT is the commonly applied method for evaluation of cell viability and cytotoxicity for screening the drugs. The MTT assay based on the reduction of MTT (yellow colored) and other tetrazolium dyes depends upon cellular metabolic activities due to NAD(P)H-dependent cellular oxidoreductase enzymes (Berridge et al., 2005).

The healthy and rapidly growing cells exhibit high rates of MTT reduction to formazan while the dead or inactive cells fail to do so. The final product of MTT reduction is a purple color formazan that can be easily dissolved in DMSO. Viability in the MTT assay is connected with the quantification of formazan at 540 nm which is linearly associated with the enzyme activity and indirectly the number of viable cells. High purple color intensity denotes higher cell viability while the decrease in purple color intensity signifies the reduced cell number and thus cytotoxicity of the given substance

The MTT assay is a colorimetric assay for assessing cell metabolic activity. NAD (P) H-dependent cellular oxidoreductase enzymes may, under defined conditions, reflect the number of viable cells present. These enzymes are capable of

reducing the tetrazolium dye MTT 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide to its insoluble formazan, which has a purple color. Other closely related tetrazolium dyes including XTT, MTS and the WSTs, are used in conjunction with the intermediate electron acceptor, 1-methoxy phenazine methosulfate (PMS). With WST-1, which is cell-impermeable, reduction occurs outside the cell via plasma membrane electron transport.Tetrazolium dye assays can also be used to measure cytotoxicity (loss of viable cells) or cytostatic activity (shift from proliferation to quiescence) of potential medicinal agents and toxic materials. MTT assays are usually done in the dark since the MTT reagent is sensitive to light.(44)

MTT, a yellow tetrazole, is reduced to purple formazan in living cells.A solubilization solution (usually either dimethyl sulfoxide, an acidified ethanol solution, or a solution of the detergentsodium dodecyl sulfate in diluted hydrochloric acid) is added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer. The degree of light absorption depends on the solvent.

XTT (2, 3 - bis - (2 – methoxy – 4 – nitro – 5 - sulfophenyl) - 2H – tetrazolium -5-carboxanilide) has been proposed to replace MTT, yielding higher sensitivity and a higher dynamic range. The formed formazan dye is water-soluble, avoiding a final solubilization step.

Water-soluble tetrazolium salts are more recent alternatives to MTT: they were developed by introducing positive or negative charges and hydroxy groups to the phenyl ring of the tetrazolium salt, or better with sulfonate groups added directly or indirectly to the phenyl ring.

MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium), in the presence of phenazine methosulfate (PMS), produces a formazan product that has an absorbance maximum at 490 nm in phosphate-buffered saline. The MTS assay is often described as a 'one-step' MTT assay, which offers the convenience of adding the reagent straight to the cell culture without the intermittent steps required in the MTT assay. However this convenience makes the MTS assay susceptible to colormetric interference as the intermittent steps in the MTT assay remove traces of coloured compounds, whilst these remain in the microtitre plate in the one-step MTS assay. Precautions are needed to ensure accuracy when using this assay and there are strong arguments for confirming MTS results using qualitative observations under a microscope. (This, however, is prudent for all colormetric assays.)[28]

WSTs (water-soluble tetrazolium salts) are a series of other water-soluble dyes for MTT assays, developed to give different absorption spectra of the formed formazans.WST-1 and in particular WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium), are advantageous over MTT in that they are reduced outside cells, combined with PMS electron mediator, and yield a water-soluble formazan. Finally, WST assays (1) can be read directly (unlike MTT that needs a solubilization step), (2) give a more effective signal than MTT, and 3) decrease toxicity to cells (unlike cell-permeable MTT, and its insoluble formazan that accumulate inside cells)[29]

PREPARATION OF REAGENTS

MTT stock solution: Dissolve 500 mg MTT powder in 10 mL phosphate buffer solution. Stir the solution with a magnetic stirrer for about 1 hour in the dark. Filter the sterilized solution with a 0.22 mm filter (Millipore, Ireland) and then store

it in 10-mL aliquots (50 mg/mL) at -20°C (van Merlo et al., 2011). The working solution (5 mg/mL) will be prepared on the day of experiment by dilution.

1. Cells were seeded in a 96-well flat-bottom microtiter plate at a density of 1 × 104 cells/well and allowed to adhere for 24 hours at 37°C in a CO2 incubator.
2. After 24 hours of incubation, culture medium was replaced with a fresh medium.
3. Cells were then treated with various concentrations of the desired compound for 24 hours at 37°C in a CO2 incubator.
4. After 24 hours of incubation, culture medium was replaced with a fresh medium.
5. Subsequently, 10 µL of MTT working solution (5 mg/mL in phosphate buffer solution) was added to each well and the plate was incubated for 4 hours at 37°C in a CO2 incubator.
6. The medium was then aspirated, and the formed formazan crystals were solubilized by adding 50 µL of DMSO per well for 30 min at 37°C in a CO2 incubator.
7. Finally, the intensity of the dissolved formazan crystals (purple color) was quantified using the ELISA plate reader at 540 nm.

General procedure:

3[4, 5-dimethylthiazol-2-yl] 2, 5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells

After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0c for 4 hr. The medium with MTT was then discarded and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The percentage cell viability was then calculated with respect to control as follows

% cell viability= [A] Test / [A] control×100

Group I	Cells Alone	1.352	0.812	1.105	1.089	100%
Group II	Cells + Etoposide	0.827	0.767	0.760	0.784	28%
Group III	Cells + sample A (500mg/ml)	0.414	0.469	0.436	0.439	59.6%

Note:

- Sample: Sample A
- Etoposide used as a standard drug
- Group I as Control (ie, Cells alone)

:

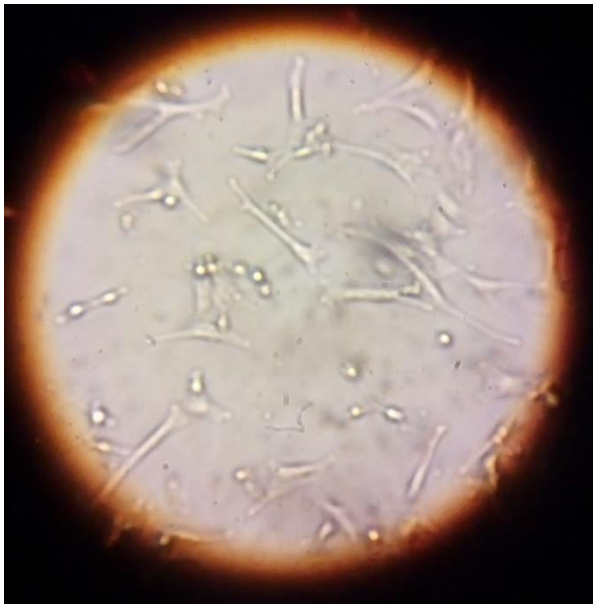


Fig. 11 : Cell line image: A 549 (Lung cancer)

The MTT assay was done and Etoposide used as the standard drug. The above table shows the result of the study. . The MTT assay is a colorimetric assay for assessing cell metabolic activity. NAD (P) H-dependent cellular oxidoreductase enzymes may, under defined conditions, reflect the number of viable cells present. . MTT assays are usually done in the dark since the MTT reagent is sensitive to light. Here we are using etoposide as the standard drug and the result compared with the cell with sample result. The assay result of cell with the standard drug etoposide shows 28% here, the standard shows 28% and the sample shows 59% there is a great difference between the results. The alcoholic extract of *Physalis lagascae* has anti-cancer activity but its less when compared to the standard drug etoposide

9. RESULT AND DISCUSSION

The result of present study, the qualitative analysis of ethanolic extract of *Physalis lagascae* showed the presence of carbohydrates, alkaloids, saponins, tannins, phytosterols and steroids and flavonoids. The effect of alcoholic extract of *Physalis lagascae* for its anti-cancer activity is tested by theMTT assay. The MTT assay is a colorimetric assay for assessing cell metabolic activity. NAD (P) H-dependent cellular oxidoreductase enzymes may, under defined conditions, reflect the number of viable cells present. . MTT assays are usually done in the dark since the MTT reagent is sensitive to light. Here we are using etoposide as the standard drug and the result compared with the cell with sample result. The assay result of cell with the standard drug etoposide shows 28% here, the standard shows 28% and the sample shows 59% there is a great difference between the results. The alcoholic extract of *Physalis lagascae* has anti-cancer activity but it's less when compared to the standard drug etoposide

When we come to the chromatographic studies.Here we used the Ethanolic extract of *Physalis lagascae*for the HPTLC. The garlic acid standard and Quercetine standard as a reference marker. The movement of the sample was compared with the other two components and finally decide the presence of compound

Yellowish orange coloured zone at Visible mode/ Blue color fluorescent zone in UV 366nm mode was present in thetrack, it was observed from the chromatogram after derivatization, which maybe the Presence of Quercetinin the given standard.

Gallic acid standard was not moved from the loading point for the given mobile phase.

Quercetin, a plant flavonol from the flavonoid group of polyphenols, is found in many fruits, vegetables, leaves, and grains; red onions and kale are common foods containing appreciable content of quercetin. It has a bitter flavor and is used as an ingredient in dietary supplements, beverages, and foods. Quercetin is a flavonoid widely distributed in nature.[2] The name has been used since 1857, and is derived from quercetum (oak forest), after Quercus. It is a naturally occurring polar auxin transport inhibitor. In plants, phenylalanine is converted to 4-coumaroyl-CoA in a series of steps known as the general phenylpropanoid pathway using phenylalanine ammonia-lyase, cinnamate-4-hydroxylase, and 4-coumaroyl-CoA-ligase. One molecule of 4-coumaroyl-CoA is added to three molecules of malonyl-CoA to form tetrahydroxychalcone using 7, 2′ -dihydroxy-4′ -methoxyisoflavanol synthase. Tetrahydroxychalcone is then converted into naringenin using chalcone isomerase.

10. CONCLUSION

The result of the shows the presence of compounds like carbohydrates, alkaloids, saponins, tannins, phytosterols and steroids and flavonoids. The MTT assay and the Chromatographic studies give more information about the drug. When we go through the MTT assay it showed that the ethanolic extract of *Physalis lagascae* showed the in vitro anti-cancer activity but it's very less when we compare it to the standard drug like the etoposide. The cell with standard drug etoposide showed 28% and the cell with sample showed 59.6%.The results can be analyzed as the cell with sample showed value higher than the cell with standard drug produced. The MTT assay gives clear idea about the in vitro anticancer activity of the plant extract.

The chromatographic study showed that the presence of the compound Quercetine which belongs to the group of flavonoids in the ethanolic extract of the plant *Physalis lagascae*. The chromatographic study showed that the standard reference marker Quercetine just moved from the initial point and the garlic acid extract didn't show any movement from the initial point. This said that the sample contain Quercetine which belongs to the flavonoid group. The importance of flavonoid is it has the property of anti-oxidant, which is very helpful in the mechanism of anti-cancer activity.so, we can say that the extract of plant *Physalis lagascae* shows the anti-cancer activity

When we analyze both the result the MTT assay shows the plant extract of *Physalis lagascae*has anti-cancer action .and the chromatographic study shows that the extract contain Quercetine which belongs to the group of flavonoids which having the property of anti-oxidant action which support the anti-cancer action of the plant

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