Available Online at www.ijpscr.info
International Journal of Pharmaceutical Sciences and Clinical Research 2023; 3(3):116-133

REVIEW ARTICLE

Advances in the Management of Cancer by Natural Products: An Overview

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Received: 10-04-2023; Revised: 28-05-2023; Accepted: 10-06-2023

ABSTRACT

Drugs derived from natural origin are often derived from medicinal plants. The extract thus obtained may be from a single plant or polyherbal, that is, a combination of botanical extracts that are assumed to have additive or synergistic effects, although some antagonistic action has also been reported. It is documented that the majority of terrestrial and marine plants have still not been fully explored regarding their prophylactic or therapeutic properties. As per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals and Institutional Animal Ethics Committee, in the absence of preliminary toxicity or activity data, the direct use of experimental animals in sacrificial/ painful models is also not fully justified. A cell line is defined as a permanently established cell culture that will proliferate indefinitely when given an appropriate fresh medium and space. Cell lines consist of cells with a uniform genetic makeup because they developed from a single cell and their subsequent growth occurs in a favorable artificial media or environment. Cell lines differ from cell strains in that they become immortalized. According to the World Health Organization, developing countries like India need to fully explore the potential of their traditional drugs in the management of various diseases, either in prophylaxis or therapeutics, to become economically self-reliant. Cancer is reported as the second primary cause of death globally; nearly one in six deaths is caused by cancer disease. Applying cancer models on experimental animals leads to a painful death. It was, therefore, thought worthwhile to compile and critically analyze the major published studies, where an herbal extract or drug of natural origin has been tried on cell lines, particularly on cancer cells, as an alternative to experimental animals.

Keywords: Anticancer, Ayurveda, Biomarker, Cancer, Cell line, Medicinal plants

INTRODUCTION

Cancer is defined as a disease in which abnormal cells divide in an uncontrolled manner and the proliferation of cells occurs in an uncontrolled manner without any differentiation process. They have some characteristics such as they invade and destroy the surrounding cells and tissues. It has been reported that some cancerous cells get detached from the main site of origin and they reach other sites distant from the original tumor by traveling through blood and lymph and forming fresh colonies, which is known as metastasis or secondary growth.

Mortality due to cancer is about 3500/million population annually around the world. Due to the tumorigenic nature of a cell, the behavior of the cell changes, and these behavioral changes such as uncontrolled growth and proliferation lead to mesenchymaltransition.[1]

Cancer is known as a fatal disease because it can metastasize to various organs. Uncontrolled

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proliferation of cells occurs in cancer, which is initiated by several factors such as mutations, genetic instability, and environmental factors. Approximately 90% of cancer-related deaths occur due to the direct or indirect effects of metastatic dissemination.[2]

It was reported by various researchers that cancer is a large group of diseases that can start in almost any organ or tissue or cells of the body where abnormal cells grow in an uncontrolled manner. In the advanced stage of cancer, cells infringe on their boundaries invade adjoining parts of the body, and spread to other organs. Metastasis is defined as the movement of cancerous cells from one place (at the origin place) to another place and it is a major cause of death from cancer. Neoplasm and malignant tumors are other common names for cancer.[3]

HISTORICAL PERSPECTIVE

Cancer diseases were not unknown in the ancient era; ancient surgeons of India knew the knowledge and management of the disease. Cancer word is not found in the Sanskrit equivalent, that is, in Ayurveda literature, the disease signs and symptoms under cancer are described in different contexts. The cancer word is derived from the Greek meaning "CRAB," which has been used in medical science as a term "CANKER" for a long duration.

WR Belt has remarked that the terminology of cancer is used as claws of crabs' means that it is not shown separate from each other; ancient clinicians were not unaware of the malignant disease. It is seen clearly in Atharva Veda.[4] Swelling or lumps situated in deeper structures or known as chronic ulcers. Such swellings or lumps have been categorized under the heading of "ARBUDA" these days, the diagnosis of cancer depends on DOSHA theory.[5]

A detailed study of the above disease is known throughout the world in the bibliography, that is, Susruta Samhita, Vangbhata, Charaka, Samhita, and Madhavanidana. Therefore, in the ancient era, it was well known for symptoms and medicine, which are described in the above-cited paragraph.[6]

Cancer is the second opinion cause of death globally, with global information, an estimated 9.6 million deaths, or one in six deaths was caused by cancer in 2018. Lung, prostate, colorectal, stomach, and liver cancers are the most common types of cancer to be present in men's bodies, while breast, colorectal, lung, cervical, and thyroid cancers are the most common among women.[7] Cancer's economic burden continues to grow worldwide, exerting tremendous physical, emotional, and financial strain on individuals, families, communities, and health systems.

The larger context of health systems in low- and middle-income countries is not a good condition to manage this burden, and in these circumstances, large numbers of cancer patients globally do not attain timely quality diagnosis and treatment. In other countries where health systems are strongly affected, survival rates of many types of cancers are improving thanks to accessible early detection, quality treatment, and survivorship care.[8] The WHO estimates that about 70% of cancer deaths occur in low-and middle-income countries (LMICs) and, it assumes that by 2030, LMICs are expected to bear the brunt of the expected 24.1 million new cancer cases per year. It is also reported that the social and economic burden of cancer in many LMICs, reducing global cancer and non-communicable diseases (NCD) burden is a prerequisite for addressing social and economic inequities, stimulating economic growth, and accelerating sustainable development. In collaboration with the NCD Alliance, UICC engaged in a 6-year advocacy campaign to position NCDs in the Sustainable Development Goals (SDGs).[9] This yielded several important wins within SDG 3. In September 2015, the global community came together at the UN General Assembly to adopt Agenda 2030 for Sustainable Development (link is external), or the SDGs.[10] This agenda is built on the successes of the Millennium Development Goals, but takes a much broader scope, recognizing the importance of integrated and indivisible action on economic growth, social equity, and environmental protection to deliver sustainable development for all.[11]

First time, the global development agenda also recognized that NCDs, including cancer, constitute major health and development challenges,

impacting every aspect of sustainable development. Target 3.4 reduces by one-third premature mortality from NCDs through prevention and treatment and promotes mental health and wellbeing.

Target 3.8 achieves universal health coverage, including some parameters such as financial risk protection, access to quality essential healthcare services, and access to safe, effective, quality, and affordable essential medicines and vaccines for all.

3A. It strengthens the implementation of the World Health Organization (WHO) Framework Convention on Tobacco Control in all countries, as appropriate.

3B. Support the research and development of vaccines and medicines for communicable and NCD that primarily affect developing countries, providing access to affordable essential medicines and vaccines.

3C. It substantially increases various parameters such as health financing, recruitment, development, training, and retention of the health workforce in developing countries, especially in the least developed countries and small island developing states.

NCD including cancer is emerging as a major public health problem in India. The disease is lifestyle-related, has a long latent period, and needs specialized infrastructure and human resources for treatment, based on the cancer registry data; it is estimated that there will be about 800,000 new cases in India every year. Cancer sites associated with tobacco from 35 to 50% of all cancers in men and about 17% of cancers in women. The main risk factor for the development of cancer is the consumption of tobacco and alcohol, consumption of a large amount of red chilies, eating food that is cooked at a very high temperature, obesity, and eating red meat, etc., in India.[12]

Tuibur has been linked to the high rate of stomach cancer in Mizoram due to the high consumption of tobacco. In south India, there is a trend toward increasing consumption of red meat and this can lead to an increased risk for large bowel cancer. Cancer of uterine cervix cancer is still the most common cancer among women in India. Therefore, India has formulated a National Cancer Control Program for the prevention and treatment of cancer. Knowledge, Attitude, Practice, and Pattern has introduced a big role in the field of cancer prevention. Anti-tobacco programs also play a major role in the above-cited area. The district hospital in India is being used as a "Cancer Detection and Prevention Clinic" which provides diagnostic services and minimal treatment.[13]

It is reported that, in India, more than 75% of cancer cases are in the advanced stage and initial stage detection is not an easy task, due to that palliative care and relief from pain are essential to making cancer patient's life better. Major regional cancer centers, medical college hospitals, district hospitals, sub-district hospitals, etc., play an important role in the care of cancer patients, but due to too many cases these alternatives are not adequate, and the service for prevention regarding cancer is useless due to the following reasons, which laid down in socially, economically, that is, poverty, shortage of food, unemployment, high price treatment, and create concerned for meeting out the cancer treatment.

The government has also introduced the AYUSHMAN Bharat Yojna under PMJAY (PradhanMantri Jan AarogyaYojna), it is the AB-NHPS (Ayushman Bharat National Health Protection Scheme). In this scheme, Ayushman cards are given to BPL as per Census 2011, for 2nd and 3rd class disease, that is, cancer, knee surgery, etc. Due to a lack of expert human resources in India or other developing countries, on the other hand, UICC (International Union Against Cancer) or other international agencies are supporting fellowships and India can utilize these opportunities to get the much-needed human resources for the country. Not only in India, it is a global issue to catch the easy solution due to unaware Red Tepism, costly treatment, and the Animal Protection Act can also be bound to use the living animal for testing, these are vital challenges in the field of cancer treatment.[14]

There is a different treatment regimen which is widely used but has side effects, such as surgery, chemotherapy, radiation therapy, immunotherapy, targeted therapy, stem cell transplantation, and hormonal therapy. In chemotherapy, different cytotoxic drugs are used to kill cancer cells

but affect healthy cells also. The objective of chemotherapy is to do maximum damage to cancer cells while causing minimum damage to healthy tissue. Chemotherapy is used a lot nowadays because it eliminates cancer and reduces the ability of cells to generate. Another therapy is radiation therapy, in that radiation is used for the treatment of cancer, and it works by damaging the DNA within cancer cells and destroying their ability to reproduce. It was reported that when the damaged cancer cells are destroyed by radiation, it eliminated naturally from the body. Radiation therapy also affects the normal cells, but they can repair themselves. Radiation therapy may be used in two different paths, by treating cancer and by reducing symptoms.[15]

Immunotherapy works by targeting cancer's specific genes, proteins, or the tissue environment that are responsible for cancer growth and survival. Inhibition of signal transduction is reported as the most common targeted therapies and they block the signals that are responsible for the fastest growth of cells.

Stem cell transplant therapy is used to help people with leukemia and lymphoma, this replacement of bone marrow is done which is destroyed by cancer or by chemotherapy or by radiation therapy. Another therapy is hormonal therapy which slows or stops the growth of cancer that uses hormones to grow, and in this therapy, some medicine is taken that blocks the activity of the hormone or stop the body from making hormone. For example, estrogen/progesterone therapy reduces the risk of colon cancer.[16]

However, above all, therapies are costly and harmful. Therefore, despite these cited concerns, many oncologists have started to use cell lines and medicinal plants for testing, diagnosis, and treatment of cancer. The cell line model is widely used for research purposes because the animal model or using the animal for research purposes is prohibited by CPCSEA and the Animal Cruelty Act. Cell lines are having great importance and promising future in the management of cancer, thereby preventing experimental animals from undergoing unnecessary trauma. Therefore, the cell line is cultured and maintained for research purposes may be considered as one of the best available model, as it prevents unnecessary usage and mortality of experimental animals.[17]

CELL LINE

The cell line is defined as a permanently established cell culture, and the cell culture technique was started in the late 19th century. After the cell culture technique, the tissue culture technique developed, that is, tissue culture within the body. A scientist Dr. Jones diagnosed a lady named Henrietta Lacks (HeLa) who was suffering from cervical cancer through the process of cervical biopsy, and he sent a sample to Dr. Gey who cultivated that sample cell and discovered that the cell line was found to be remarkably durability and cell diving property, and this new cell line called as HeLa cell line. These cell lines quickly reproduced in almost any media.[18]

Cancer cell lines (CCLs) were initially derived from tumors and cultured in a two-dimensional environment, and large-scale genomic profiling combined with existing genomic data allows the evaluation of the utility of cell lines as models for metastatic cancer. In general, cancer cells would divide a few times and die off before any decent studies could be done with them, but Lady Henrietta had a great property of dividing and dividing, and that cancer cells became the first human cell line to be established in culture and Gey named that cell line as HeLa. Gey distributed these cell lines to several laboratories and pharmaceutical companies, making this cell line a great resource for cancer research. Nowadays, various cell lines are used for research purposes such as MCF-7, PC-12, PC-3, HEK-293, HL-60, and Vero cell line.[19]

It is defined as a permanently established cell culture that will proliferate indefinitely given appropriate fresh medium and space. Cell lines differ from cell strains in that they have absconded the Hay flick limit and become immortalized. The cell line arises from a primary culture at the time of the first successful subculture. The cell lines may be propagated for a limited number of generations beyond which they may die off or give rise to a continuous cell line, and a continuous cell line is an aneuploidy in nature with

a large amount of variation in chromosome number while the finite cell lines are euploid with a little variation in the number of chromosomes. A culture is transformed by a process of *in vitro* transformation and converted into a continuous cell line, and this transformation may be induced by viruses or some chemical reactions. There is some reason why continuous cell lines are called transformation ones, because the culture in terms of morphological, as well as the kinetic alterations that occurred, here it should also be stated that, the cell lines are more prevalently accompanied by an increased tendency of developing tumor. Therefore, continuous cell lines show malignant transforming properties. It is reported that continuous cell lines are having some characteristics, like they required a reduced serum level, high plating efficiency, less nutritional requirements and also exhibit aneuploidy condition in dividing cells. Continuous cell lines are usually aneuploid and often have a chromosome number between the diploid and tetraploid values. Various normal cells do not give rise to continuous cell lines.[20]

The classical example is normal human fibroblasts that remain euploid throughout their lifespan and in crisis (usually around 50 generations) will stop dividing, although they may remain viable for up to 18 months thereafter. Human glia and chick fibroblasts behave similarly. Some characteristics of cell lines that make these cells unique and biomedically or biotechnologically useful, including their growth pattern, determined morphological appearance of the cell line, and should be stable from the master cell bank to the end-of-production cells. If chromosomes and specific surface markers are useful in characterizing the cell line, then these markers should be characterized for stability. Mostly cultured cell lines are allowed to generate their extracellular matrix (ECM), but primary culture and propagation of some specialized cells, exogenous provision of ECM. Many transformed cell lines provided the best model for the induction of differentiation. It was found that normal cells have limited dividing capacity; therefore, cell lines derived from normal tissue remove out or die out, after a fixed number of population doublings, and it is a genetically determined event that involves different genes.

Many human continuous cell lines show a common feature of the development of a subtetraploid chromosome number. *In vitro* transformation occurs due to alteration in a culture that gives rise to a continuous cell line and may occur spontaneously or be chemically or virally induced.[21]

For the maintenance of the cell line, some basic conditions are required, like.

pH

It was found that most of the cell lines grow well at pH 7.4. Although the optimum pH for cell growth varies relatively little among different cell strains, some normal fibroblast lines perform best at pH 7.4 to pH 7.7, and transformed cells may do better at pH 7.0 to pH 7.4.

Buffering

Culture media must be buffered under two sets of conditions:

a. Open dishes, where the evolution of CO_2 causes the pH to rise
b. Overproduction of CO_2 and lactic acid in transformed cell lines at high cell concentrations, when the pH falls.

Temperature

The temperature that is recommended for most human and warm-blooded animal cell lines is 37°C, which is close to body heat, but commonly we set a little lower temperature for safety purposes, because overheating may become a major problem than under heating.

Media

Although numerous cell lines are still propagated in a medium that is supplemented with serum, in many instances, cultures may now be propagated in serum-free media. Commercially produced media will have been tested for their capability of sustaining the growth of one or more cell lines. However, under certain circumstances, we can use our media.

Growth curve

There are mainly three parameters that are measured by the growth curve,

1. The lag phase that is before cell proliferation is initiated after subculture, and indicating whether the cells have to adapt to various conditions;
2. The doubling time, that is in the middle of the exponential growth phase, and indicating the growth-promoting capacity of the medium;
3. The maximum cell concentration attainable indicating whether there are limiting concentrations of certain nutrients.

In cell lines whose growth is not sensitive to density (e.g., continuous cell lines), the terminal cell density indicates the total yield possible and usually reflects the total amino acid or glucose concentration.[22]

Generation of cell lines

Stably transfected cell lines are used extensively in drug discovery. Cell lines that express a target of interest, such as a G-protein coupled receptor or a reporter gene, form the basis for most cell-based compound screening campaigns. In establishing new assays for high-throughput screening, the creation of the appropriate cell line is a bottleneck. Typically, a stable cell line is created by transfection with a plasmid encoding the target of interest or a reporter gene construct, and an additional gene that allows for the chemical selection of successfully transfected cells (usually an antibiotic resistance gene).[23]

CELL LINE GENERATION

The desired stable cell line is generated through a lengthy selection process and subsequent limiting dilution to obtain clones. It was reported that this process takes approximately 2–3 months and usually yields 5–10 usable clones allowing little control over the results throughout the process. Laser-enabled analysis and processing (LEAP) technique for cell line generation has been developed for high-throughput laser-mediated cell elimination for cell purification based on fluorescent and morphological criteria. Selection of cells of interest by the LEAP method was done that is based on various factors such as fluorescent properties, morphological properties, or a combination of both.

Antibiotic resistance genes are replaced by chemical selection with a gene encoding a fluorescent protein, transfected cells can be selected based on fluorescence. These cells can be purified using the LEAP method specifically eliminating non-fluorescent cells using laser technique. By selecting cells that remain fluorescent and proliferate over some time, stable cell lines are isolated. Besides, the fluorescence levels may be used to identify cell lines with a specific desired expression level of the transfected construct. Several different types of cell lines can be finite or continuous, prepared from normal tissue as well as neuroplastic tissues. The cell bank system is generally used for the maintenance of cell lines with a constant supply of starting material as well and it performs cell line characterization and detection of cell line cross-contamination. In drug discovery, stably transfected cell lines are generally used.[24]

NOMENCLATURE OF CELL LINE

Cell line naming is done as a code or designations for their identification, for instance, the code NHB 2–1 represents the cell line from the normal human brain, followed by cell strain or cell line number 2 and clone number 1, while naming of the lines it is required that ensures that the cell line designated is unique so that there is no confusion when it is given in the literature, and at the time of the publication that cell lines should be prefixed with a code designating the laboratory form like National Cancer Institute (NCI) for the NCI.[25]

APPLICATION OF CELL LINE

It was found that approx. 3600 cell lines are available that have been developed from different biological species (approximately 150), including the mouse Sertoli cell line (MSC-1), Hepa16 cell line, HeLa cell line, and clonal cell line. Cell lines

are ethically viable resources for scientific research and it is cost-effective. The cell line is used as a role model due to their phenotype and genotype homogeneity over cell populations. They are used in different fields such as antibody production, vaccine development, drug testing, cancer cell growth studies, genetic function studies, and artificial skin development.

It is also used for the development of a biomarker for cancer, lung dysfunction, and better visualization of tumor cells, and for better results, cell line engineering methods are applied. However, some growth conditions are required for 3D cell culture development such as polystyrene mats, polycarbonate-based ECM fibers, and collagenous mats. Cell line/cell culture was used for toxicity testing, as a model system for studying basic cell biology, effects of drugs on cells, process and triggering of aging for nutritional studies, and also in gene therapy.[26]

SELECTION OF CELL LINES

There are some criteria for selecting the appropriate cell line for any research work/experiments; such as species(non-human cell lines preferred because they have less risk of biohazards), functional characteristics (liver and kidney-derived cell lines are more suitable for toxicity testing), and culture with continuous cell lines are preferred because they grow faster, easily cloned, and maintained. Moreover, the productivity is high, transfected cells are preferred as they are immortalized and grow rapidly. However, the growth criteria of the cell lines are most important, and it is necessary to consider other factors such as population doubling time, ability to grow in suspension, saturation density, and cloning efficiency.

Nowadays cell lines play an important role in studying the various phenomena such as physiological, pathophysiological, and differentiation processes of specific cells and it also allows the examination of stepwise alterations in the structure, genetic makeup, and biology of the cell under controlled environmental conditions. Nowadays, cancer cell lines (CCLs) are one of the most widely used experimental models to understand cancer biology, as well as to test the efficacy of novel anticancer therapies. It was found that the human embryonic kidney cell line (HEK293) and the Chinese hamster ovary cell line are used as excellent host cells for robust secretion of mammalian proteins with appropriate posttranslational modifications. Stable cell lines require substantial time and effort in comparison to transient transfection processes that were established. An expression vector with the gene of interest has to be inserted into the host cell genome. Generation of a stable cell line occurred from transfected cells that have integrated the vector into their genome. It was reported that stable cell lines are responsible for the overexpression of the target protein uniformly and indefinitely.[27]

There is one example is the use of three-dimensional (3D) cell culture models which could be non-adherent (anchorage-independent) or adherent to a substrate (anchorage-dependent). In the 3D anchorage-independent culture, the aggregation of cells can be achieved using low-attachment plates and through coating surfaces (e.g., poly-hydroxyethyl methacrylate and agarose). In the anchorage-dependent model, a 3-D environment can be established with the use of pre-fabricated scaffolds, which consist of porous materials to support the growth of 3D structures called multilayered cell cultures which result from cells adhering to specific substrates that are composed of tumor cells cultured on a membrane specifically designed to allow measurement of drug diffusion.[28]

It was found that microfluidics channels were also able to support the formation of 3D cell cultures; ECM can also be added into these chambers to allow ECM-to-cell interactions. Exploiting cells grown in 3D culture conditions has some advantages like oxygen and nutrient gradients, cell-to-cell interactions increased, different rates of cellular proliferation, and more. The primary goals for developing 3D cell culture systems vary widely from engineering tissues for clinical delivery to the development of models for drug screening.[29]

Tissue engineering certainly one of the most promising developments in the applications of cell culture is the development of tissue engineering, through which it seeks to develop and establish cell growth *in vivo* so that in the future to be used

as replacement tissue damaged or malfunctioning of the patient requires it. Thus, tissue engineering is now considered an end product for regenerative medicine as well as enabling technologies for other fields of research ranging from drug discovery to biorobotics 3D tissue models for drug testing (to evaluate toxicity anticancer drug efficacy, cellular immunity by cytokine release, monitoring events such as proliferation and apoptosis, among others, and therapeutic drug testing), development of multicell type artificial cornea mimics.[30]

MEDICINAL PLANTS

Definitions

Medicinal plants are defined as any plants that contain therapeutic properties or compounds (phytochemicals) that can be used for the management of a disease or those that synthesize metabolites to produce useful drugs, according to the World Health Organization (WHO), (WHO 2008). According to the WHO, approximately 65–80% of developing countries depend on traditional medicine for their health care due to difficulties in accessing modern medicine or poverty. Phytochemicals/biologically active compounds present in biological systems, and it is non-nutritive plant chemicals that have protective or disease-preventive properties. These phytochemicals play that some important functions such as acting as an antioxidant, antibacterial effect, and stimulation of enzymes, show some hormonal action, etc.[31]

Cancer is one of the most serious diseases that affect the duration and quality of an individual's life all over the world. It was found that conventional therapeutic strategies fail to fulfill the major requirements for successful cancer therapy, and due to that, the use of naturally developed anticancer agents has evolved as an alternative, safe, low-cost, and convenient one. There are a huge amount of medicinal plants, used traditionally for thousands of years, that are present in the herbal preparation group of the Indian traditional health-care system (Ayurveda) named Rasayana proposed for their interesting antioxidant and anticancer activities.[32]

In the ancient era, Egyptian civilization left a wealth of information on medicinal plants and medical practices. The Ebers papyrus from ancient a prescription for cannabis applied topically for inflammation. This papyrus is the most important and complete medical papyrus recovered, containing 110 pages (700 magical formulas and folk remedies). Nowadays, scientists started purifying active extracts from some medicinal plants, for example, Serturnerisolates, and morphine from opium poppy, which was a great work in the field of pharmaceutical chemistry. However, direct use of plant extracts continued to decrease in the late 19th and 20th centuries. Today, medicinal plants still contribute significantly to prescription drugs, but 25% of prescriptions written in the U.S. Natural therapies, such as the use of plant-derived products in cancer treatment, may reduce adverse side effects.

At present, it is found that few plant products are being used for the treatment of cancer. However, a myriad of plant products exist that are reported to have very promising anticancer properties *in vitro* but have yet to be evaluated in human beings.[33]

Anticancer properties of medicinal plants have been known for a long time or centuries. Podophyllotoxin and several other compounds that are known as lignans isolated from the *Podophyllum peltatum* plant, commonly known as common mayapple, ultimately led to the development of the drug. NCI has screened approximately 35,000 plant species for potential anticancer activities.

Various medicinal plants are considered a rich source of a wide variety of ingredients (phytochemicals) that can be used for the development of herbal drugs. It is well-known that cancer is one of the dead diseases which are characterized by irregular cell proliferation, uncontrolled cell growth, etc., and the treatment of that is very costly and harmful. It is a major health issue in developing and developed countries.[34]

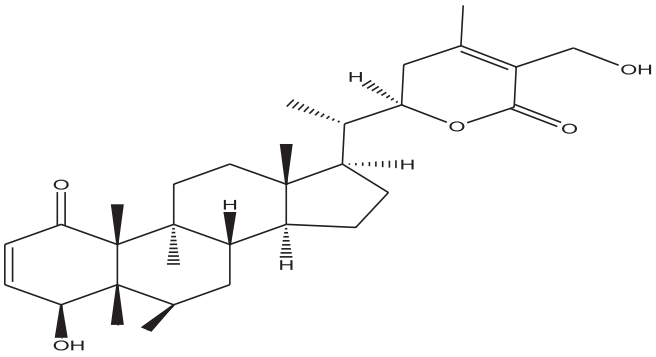
High mortality and incidence make cancer an important public health and economic issue that requires effective treatment and prevention methods. There are various treatment therapies such as radiotherapy, immunotherapy, and chemotherapy used for cancer treatment, but these techniques adversely affect healthy cells along with cancer cells. Compounds that are isolated

from medicinal plants have some advantages over synthetic chemical compounds as they are readily available in nature, their cost is low, toxicity is less, etc. Due to these properties, medicinal plants are used as an alternative treatment for cancer.

The anticancer properties of several plants are still being actively researched and some of them have shown promising results. Various plants and plant products are reported that have a promising anticancer activity that is discussed in detail below:-

Andrographis paniculata

Andrographolide is a major chemical constituent of *A. paniculata*, is a diterpene, and is responsible for its anticancer activity. Andrographolide is called "diterpene lactone" due to its ring-like structure. It has a bitter taste and shows a colorless crystalline appearance. It was reported that when the extract of A. paniculata was studied on mice, it was found that it is a stimulator of the immune system and is responsible for the activation of both types of immune response, antigen-specific, and non-specific immune responses.



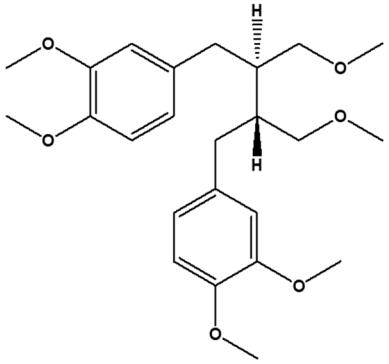
Chemical structure of Andrographolide A

A. paniculata is reported as a potent anticancer agent and is effective against a variety of infectious and oncogenic agents due to its ability to activate both types of immune responses. Andrographolide shows cytotoxic activity against a variety of cancer cells.[35] For example, andrographolide exerts cytotoxic effects on various CCLs, human epidermoid cancer cells (KB), lymphocytic leukemia cells (P388l), breast cancer cells (MCF-7), colon cancer cells (HCT-116), etc. Andrographolide, a major chemical constituent of the A. paniculataplant, has also shown a significant anticancer and immunostimulatory activity.

It shows cytotoxic effects against breast cancer cells (MCF-7), P388 lymphocytic cells, and colon cancer cells (HCT-116). Andrographolide shows inhibition of growth in colon CCLs HT 29 and enhances growth and division of human peripheral blood lymphocytes in mouse myeloid leukemia M1 cell lines.[36]

Phyllanthus amarus

P. amarus contains various lignans, flavonoids, and tannins, and evidence suggests that *P. amarus* extract may exert antitumor effects. Oral administration of *P. amarus* extract significantly increased the lifespan and reduced tumor size in mice bearing Dalton's lymphoma ascites and Erlich ascites carcinoma. The extract of *P. amarus* has been shown to inhibit aniline hydroxylase, a P-450 enzyme responsible for the activation of *carcinogens*.



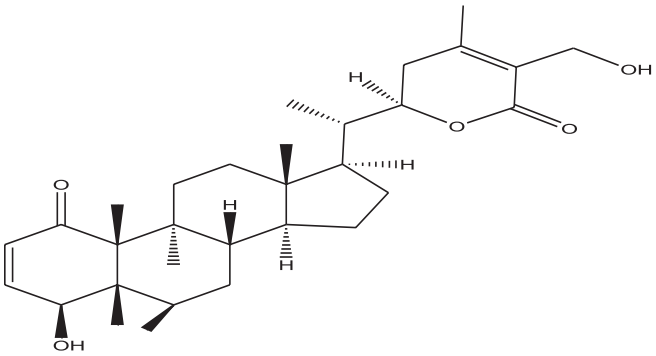
Chemical structure of phyllanthin

It was reported that when the extract of *P. amarus* was tested on various cancer cells, it inhibited CDC 25 tyrosine phosphatase activity, which is a key enzyme involved in the regulation of the cell cycle. It shows anticancer activity, due to the ability to induce cell cycle arrest, interfere with DNA repair, and metabolic activation of carcinogenic compounds inhibited. *P. amarus* extract also showed antiangiogenic effects when tested on mice that are suffering from Lewis lung carcinoma, the migration of vascular endothelial cells is interfered with.[37]

Withania somnifera

W. somnifera plant, due to its energy-promoting nature, stress-relieving benefits from ancient

times and mentioned in the Indian traditional system of medicine. It was found that withanolide A, which is a major constituent in the root of this plant upregulated the Th1-immune response. Withaferin A, another chemical constituent of *W. somnifera*, is distributed mostly in leaves and produces rapid apoptosis in cancer cells. Leaf extract at 150 mg/kg was highly toxic to mouse Sarcoma-180, Ehrlich, and ascites tumor models, while a comparable dose of *W. somnifera* formulation was highly effective in producing tumor regression by >50%; the root extract, however, was relatively poor in tumor regression.[38]

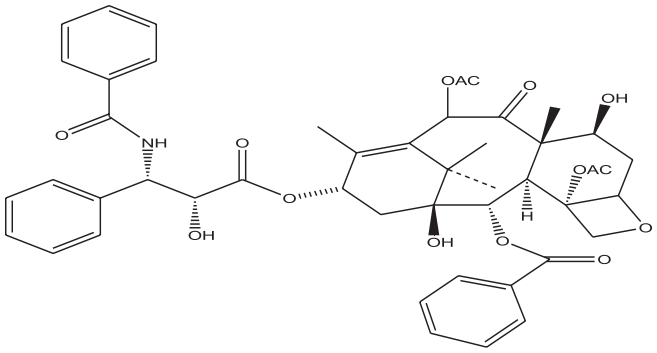


Chemical structure of Withaferin A

The presence of a high content of withaferin A in this plant is responsible for anticancer activity by cell signaling pathways. Formulation of *W. somnifera* showed induction of cell cytotoxicity in various human CCLs. *W. somnifera* formulation also upregulates the population of T-cell population in mice (bearing tumor) with increased expression of IL-2 and IFN-gamma levels.[39]

Taxus baccata

The Pacific yew tree (*Taxus brevifolia Nutt.*) was the first plant species to demonstrate anticancer properties. These properties were isolated in very low concentrations from extracts found in the bark of the Pacific yew, which contains a compound called paclitaxel; like all taxanes, paclitaxel was determined to be toxic to cancerous cells. The yew (*T. baccata*), particularly the Pacific Yew, *T. brevifolia*, is employed for its taxol content, which is being used very successfully as a chemotherapy treatment for breast and ovarian cancer.



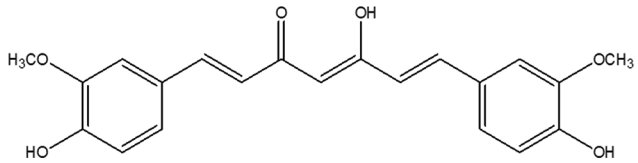
Chemical structure of Taxol

A diterpene amide, taxol isolated from the *T. brevifolia* (Pacific yew) plant, is reported as a novel anticancer agent and it is having significant activity in the treatment of various cancers such as lung cancer and malignant melanoma solid tumors.[40] Taxol acts as an anticancer agent by inhibiting the replication process induces polymerization of tubulin, inhibits disassembly of microtubules, etc. It shows anticancer activity in breast cancer xenografts.[41]

Curcuma longa

Curcumin is isolated from the *C. longa* plant which is a polyphenolic compound that is used for wound healing, skin, gut diseases, etc. It was reported that curcumin has a wide range of beneficial properties, including relieving heartburn, arthritis, anxiety, anti-inflammatory, antioxidant, chemopreventive, and chemotherapeutic activity.

It was found that when curcumin is tested against pancreatic cancer, it potentiates the antitumor effect of gemcitabine in preclinical models. Curcumin is relatively non-toxic and exhibits limited bioavailability. Curcumin acts as a potent anticancer agent by inhibiting the proliferation and invasion of cancer by cellular signaling pathways.[42]



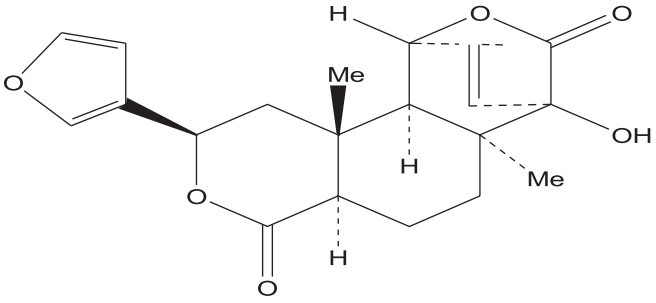
Chemical structure of curcumin

The anticancer activity of curcumin can be improved by structural modification, for example, the coplanar hydrogen donor group and β-diketone

moiety are important for the antiandrogenic activity in prostate cancer. Curcumin is currently used in human clinical trials for a variety of malignancies, including multiple myeloma, pancreatic cancer, myelodysplastic syndromes, and colon cancer. Numerous studies showed that curcumin induces apoptosis, interferes with the progression of the cell cycle, and inhibits proliferation. Curcumin also showed colon and gastric cancer prevention in rodents. Curcumin shows a protective effect by inhibiting the growth of several angiogenesis associates and tumor-associated genes.[43]

Tinospora cordifolia

It belongs to the Menispermaceae family and is commonly found in different countries such as Sri Lanka, India, and China. Various important alkaloids are found in the stems and roots of the *Tinospora* plant. It is known as "giloya" in Hindi, "guduchi" in Sanskrit, and the heartleaf moonseed plant in English. The root of this plant contains various alkaloids which include tinosporin, choline, isocolumbin, columbin, tetrahydroplamatine, magnoflorimne, and palmatin. The stem of *T. cordifolia* is commonly used for the treatment of fever, dyspepsia, jaundice, skin, and urinary disease.



Chemical structure of Tinosporin

It was reported that *T. cordifolia* can kill HeLa cells when an *in vitro* study was performed on CCL HeLa cells, and this shows the potential of this plant as an anticancer agent. *T. cordifolia* extract shows dose-dependent cell death compared to the controls. The dichloromethane extract of *T. cordifolia* showed anticancer activity in mice transplanted with Ehrlich ascites carcinoma.[44]

It was reported that medicinal plants are rich sources of herbal properties, containing phytochemicals that are responsible for the discovery of new drugs that are used in various disorders and diseases like cancer without symptoms of any toxic effects on the individuals treated. Treatment of cancer by use of natural products and traditional medicine by applying the concepts of Ayurveda is awarded a great significant scope of cancer research. This review presents the importance of traditional medicine, the anticancer properties of various natural products, and the use of medicinal plants in cancer treatment. Medicinal plants contain good immunomodulatory and antioxidant properties which lead them to be an anticancer drug. In *Tinospora*, terpenoids, anthraquinone, saponin, and phenolics are present which show anticancer properties.[45]

It was found that only a few selected plants have been explored for their biological activity from around 1000 species and many more so further investigations into the anticancer activity of the plants showing promising activity must be undertaken. Alkaloids such as Vinblastine and Vincristine, isolated from *Vinca rosea* are well-known potent anticancer drugs. It was reported that taxol was isolated from the *T. brevifolia* plant and has high anticancer potential. In nut cells, it can be concluded that cancer is the leading cause of death in developing countries like India. Compared with synthetic drugs, herbal drug treatment is less expensive so it is commonly recommended to rural and poor people to treat cancers of various types as an ideal choice.[46]

HERBAL MEDICINE IN CANCER THERAPY

Herbal medicines that are used traditionally are naturally present and plant-derived substances showing minimal or no industrial processing and have been used in the treatment of various illnesses within local or regional healing practices. Tyler defined herbal medicines as "crude drugs of vegetable origin that are utilized for the treatment of various diseases, often of a chronic nature, or to attain or maintain a condition of improved health." Herbal medicine plays an important role in the health-care system of large proportions of

the world's population, including developed and developing countries. The use of medicinal plants in various diseases is recognized and due to their economic benefits, formulations are developed that are having an increasing demand in both developing and industrialized countries.

Using this formulation or medicinal plants, toxicity from chemotherapy and radiation therapy is reduced while tumor-killing capacity is enhanced. Reduction of various side effects such as fatigue, hair loss, mouth sores, nausea, vomiting, appetite loss, and organ failure are reported when herbal formulations are used. An approach towards Prevention of cancer may be by keeping a check on spreading of tumor through growth, metastasis, and local invasion. Due consideration should also be given to offer Protection against developing cancer causing effects of radiation and chemotherapy. Care should be taken to ensure that Protection is offered against the progression or proliferation of cancer in cancer-prone individuals. Improvement of complete or partial remission by exerting direct effects on the tumor by changing the underlying conditions that allows cancer to exist. Due consideration is also to be given regarding duration and quality of life of individuals.[47]

HERBAL MEDICINES IN TRADITIONAL HEALING OF CANCER

Cancer is reported as a major cause of death and the number of new cases, as well as the number of individuals that are living with cancer, is increasing continuously. Plants have an enormous propensity to synthesize mixtures of structurally diverse bioactive compounds due to this property; the plant kingdom is used as the potential diverse source of chemical constituents with tumor cytotoxic activity. Despite the successful utilization of phytochemicals including vincristine and taxol, in mainstream cancer chemotherapy, various commercial plant-derived anticancer formulations represent only one-fourth of the total repertoire of available treatment options. It was found that during the pre-operative period, cancer patients use some herbal supplements as alternative medical therapies. It was found that approximately 25–85% of cancer patients are using herbal therapy as alternative and complementary nutritional therapy for the prevention of cancer or during the treatment of cancer.[48]

It is found that cancer is one of the major big issues in both developing and developed countries and various synthetic drugs are used for the treatment of cancer, but their use is limited due to their toxic effects on normal healthy cells along with cancer cells. Due to that, there is a large demand for alternative medicine for the treatment of cancer. Due to its safe, less toxic nature, low price, etc., medicinal plants or herbal drug formulations are used to a great extent for the management of various diseases and to maintain the health of human beings. It contains different secondary metabolites including alkaloids, flavonoids, and tannins that are responsible for potent activity toward the management of various diseases including cancer. Anti-cancer agents that are derived from medicinal plants are used to contribute to the development of new drugs. It was reported that the extracts of various medicinal plants and their secondary metabolites are responsible for anticancer activity. The discovery and development of plant-derived drugs show great promise for the future. This review contains medicinal plants with their secondary metabolites that show anticancer activity.[49]

The potential of secondary metabolites in the anticancer activity shown by *in vitro* studies and the plant metabolites mentioned in this review possess anticancer activity by various mechanisms. Therefore, in this review, various medicinal plants and their important phytocompounds were summarized that are used for the treatment of cancer. The diseased condition can be measured by the presence of some chemical compounds such as markers or biomarkers, their concentration is enhanced by the diseased conditions, so the measurement of biomarkers is useful in the prognosis/prevention of disease.[50]

BIOMARKER

It is defined as a biological molecule or naturally present molecule or genes that are found in blood, other body fluids, or tissues that are a sign of a normal or abnormal condition of the body, like

during disease condition the level of biomarkers are enhanced. For example, a cancer marker is a biomarker that can be found in the body when cancer is present.

TYPES OF BIOMARKERS

There are mostly two types of biomarkers; the first is prognostic and the second one is predictive biomarkers. The prognostic biomarker is related to the disease or cause of disease irrespective of the treatment used. These biomarkers are used to identify disease recurrence or progression in patients who have the disease or medical condition of interest. For better understanding, there are several examples such as breast cancer genes 1 and 2 BRCA1/2 mutation may be used as prognostic biomarkers when evaluating women with breast cancer, to assess the likelihood of a second-time breast cancer problem. Another one is C-reactive protein level that may be used as a prognostic biomarker to identify patients with Unstable Angina. The second is a predictive biomarker that is drug-related and they respond to a particular treatment. For example, thiopurine methyltransferase genotype or activity may be used as a predictive biomarker when evaluating patients who may be treated with 6-6-mercaptopurine or azathiopurine, to identify those at risk for severe toxicity due to high drug concentration.[51]

IDEAL PROPERTIES OF BIOMARKERS

They should be non-invasive, low cost, measurement is easy, their detection is easy, and produce rapid results. They are obtained from readily available sources such as blood and urine. They should have a high sensitivity, allowing early detection, and specific to the tumor. The abnormal level should be obtained in the presence of micrometastases and healthy individuals are at a much lower concentration.

CLINICAL USES

Biomarkers are used for various purposes such as screening, differential diagnosis, determination of risk, prediction, and monitoring of disease. The widespread use of prostate-specific antigens (PSA) in prostate cancer screening has motivated some researchers to identify suitable markers for the screening of different types of cancer. Biomarkers are also useful for diagnosis, monitoring of disease progression, predicting disease recurrence, and treatment efficacy.[52]

Different markers are present that are isolated from medicinal plants and serum. Alpha Feto Protein (AFP) formed in serum and 70 kDa glycoprotein and homologous to albumin. AFP exhibits microheterogeneity due to varying levels of glycosylation and has a half-life of approximately 5–7 days. AFP produced by malignancies is more highly fucosylated than formed in normal tissues. AFP is mainly confined to various malignancies such as germ cell tumors of the testis and ovary, hepatocellular carcinoma, and hepatoblastoma in children. AFP is proteinaceous that is normally made by fetus liver cells when the liver is immature. It was reported that abnormal levels of AFP occurred during pregnancy in some cases and there does not need to be a problem with the baby. If AFP levels are reported abnormal, it means that more tests should be performed to identify the cause of the abnormality. It is also said by the American Pregnancy Association that all pregnant women should be offered an AFP test between the 15th and 20th week of pregnancy.[53]

It was found that the AFP tumor marker test can be performed on various samples such as blood samples, urine samples, and amniotic fluid samples. Other names for the test include total AFP, MSAFP (maternal serum AFP), and alpha-fetoprotein-L3 percent (%). Alpha-fetoproteins can also play an important role in the development and progression of liver cancer. AFP regulates cell proliferation by receptor signal transportation and gene expression mechanism.[54]

PSA is formed in serum and approximately 28.4 kDa single chain, chymotrypsin-like serine protease containing 237 amino acids. PSA formed a complex with A1 antichymotrypsin, A1 antitrypsin, and A2 macroglobulin. Half-life is approximately 2.5 days. PSA is used for determining prognosis in patients with prostate cancer and in monitoring

therapy in patients with diagnosed prostate cancer. It is reported that most men with high PSAs do not have prostate cancer. Their high PSAs might be due to: An enlarged prostate gland, A prostate infection, recent sexual activity, and A recent long bike ride. Other important biomarkers such as HCG, CA125, and CA 15-3 CEA.[55]

Biomarkers related to cancer may include proteins, changes in gene mutations, rearrangements of genes, extra copies of genes, and missing genes. It was reported that different types of cancer biomarkers are available that work in a different manner in the body and react differently to treatment. Some biomarkers are reported that they trigger the growth of cells and multiplication of cells. The HER2 protein is an example of this type of biomarker, which helps to control cell growth. It was found that if HER2 protein is "overexpressed" in cancer cells, then those cells are known as "HER2-positive," meaning they produce more of the protein than is normal. In this condition, the cells grow more quickly and increase their chances of spreading to different parts of the body (metastasize). In this case, we used treatment that is responsible for the disruption of a pathway known as the HER2 signaling pathway; they help stop the growth of cancer cells.

The second one is biomarkers that support a treatment's cellular or molecular action. This type of biomarker is exemplified by a gene called SPARC, which stands for secreted protein, acidic, and cysteine-rich. SPARC helps bring albumin, a type of protein found in blood, egg whites, milk, and other substances, into cells. Some chemotherapeutic drugs are bound ("packaged") to albumin to prevent them from being dissolved in the bloodstream before they reach their target cells. Therefore, overexpression of SPARC helps treatments bound to albumin work more effectively by bringing the treatment right into the cell.

The third type of biomarker disrupts the treatment of cellular or molecular action, and some chemotherapeutic drugs are made using platinum for the disruption of the DNA of cancer cells. However, the ERCC1 protein is responsible for cancer DNA repair. However, it was found that if high levels of ERCC1 are found in the tumor patient, then platinum-based agents are not very effective for that patient. Even within the above biomarker categories, there is variety. For example, molecules that trigger abnormal cell growth can come from a gene mutation or extra copies of an otherwise healthy gene within the tumor's DNA.[56]

DETECTING AND MEASURING BIOMARKERS TO DEVELOP A PERSONALIZED ANTICANCER TREATMENT PLAN

It is most important to determine the level of certain biomarkers that are present in cancer patients, for that, a physician collects a sample of cancerous tissue or bodily fluid and sends it to the laboratory for testing at the molecular and pathological level. By these findings of the test, levels of specific biomarkers of cancer are measured and detected, after that these findings will be matched with published research data by the world's leading cancer researchers to identify which treatments are suitable for that patient. When the report contains lists of biomarkers detected in the sample received by a physician, along with the treatments, by analyzing the unique biomarker profile of that patient, the treatment of cancer patients is personalized.[57]

PHASE OF EVALUATION OF BIOMARKERS

There are a total of five phases in the evaluation of biomarkers, phase 1, phase 2, phase 3, phase 4, and phase 5. In phase 1, the identified markers are put on a priority basis which is based on their diagnosis/prognostic/therapeutic value that could suggest their evolution into routine clinical use. In phase 2 assays, promising results may further be explored for intended clinical use in near future. The assay is necessary to validate for checking reproducibility. During phase 3, the sensitivity and specificity of the test are evaluated for the detection of a disease that has yet to be detected clinically. There are some examples of biomarkers isolated or present in plants such as phenethylisothiocyanate, sulforaphane, indole-3, carbinol, crocetin epigallocatechin, lycopene, kaempferol, and

Vitamin E. These are active constituents that are present in different medicinal plants in active form and act as a biomarker.[58]

miRNAs regulate multiple gene targets and are capable of controlling a wide range of biological processes including growth, differentiation, and apoptosis. Hence, dysregulation of miRNAs may contribute to many diseases that are known to arise from an imbalance in the regulation of these cellular processes. It has been shown that mutations and disruption in miRNA biogenesis are also involved in cancer. There are some examples of biomarkers like circRNAs acting as molecular markers or potential targets providing promising application perspectives, such as in early tumor diagnosis, therapeutic evaluation, prognosis prediction, and even gene therapy for tumors. Extraction of circRNAs is easy as compared to proteins; hence, circRNAs are an ideal molecular marker for cancer.[59]

THE PROBABLE LINK BETWEEN CCLS, MEDICINAL PLANTS, AND BIOMARKERS

As we know in cancer disease, genetic and epigenetic variation occur, and there are different models used for research purposes. Cell lines are having great importance and promising future in the management of cancer compared to the animal model (animals undergo unnecessary trauma Animal Cruelty Act 1960). Before using these cell lines for research purposes, characterization of cell lines is required because it helps in the understanding of the complexity of the etiology of cancer and the different mechanisms which involved in the disease.[60]

A cell line gives information about the signaling pathway of the disease and tells about which genes are deregulated. These cell line models have great importance in cancer research because they help in the investigation of genetic and epigenetic pathways, apoptosis, and cancer progression and are used for the screening of cancer therapeutics regimens. Cell lines are easily handled and manipulated if necessary, and they have a high degree of similarity with tumor cells.[61]

Biomarkers related to cancer are used for treatment purposes because biomarkers show the status of cancer, and their growth is used for treatment purposes because biomarkers show the status of cancer, their growth pattern, and the form of cancer (Benign and malignant tumors). A combination of miR-148b, miR-409-3p, and miR-801 is used as a biomarker for differentiation in breast cancer cases and healthy people. As we know hCG is present in a pregnant ladies, but if the concentration of hCG is increased remarkably, it shows the presence of a tumor in the body, and therefore the treatment regimen may be followed upon accordingly, as per need.[62]

There are different treatment regimens for cancer management including medicinal plants which are a natural medicine used for the management of cancer from ancient times. Different medicinal plants, such as *V. rosea, C. longa*, and *T. baccata,* are used for the management of tumors. It has been documented that almost 70% of our natural drugs have still not yet been fully explored. Even authorities like WHO state that countries, particularly developing ones like India, should fully explore and utilize their native medicinal plants to become economically self-dependent. There are different phytoconstituents such as flavanoids (apigenin, kaemferol, quercetin, tangeretin), alkaloids (vincristine, vinblastine, camptothecin, teniposides, etoposide**,** berberin), and terpenes (andrographolide) used as natural anticancer agents.[63,64]

REAL-TIME CELL ANALYSIS SYSTEM FOR EXAMINING THE CYTOTOXICITY OF DRUGS TO CCLS

Intelligence real-time cell analyzer (RTCA) is used for the label-free real-time monitoring of cancer cell proliferation, viability, and cytotoxicity. In the RTCA system, 16 well microtiter plates with a gold microelectrode biosensor array are used, which measures the encumbrance when cells adhere to the microelectrodes causing an alternating current. RTCA systems may be used for the analysis of cell proliferation, viability, morphology, cytotoxicity, and migration, through the measurement of the

electric field generated in this process. This system can be used for drug discovery by measuring cytotoxicity. Using xCELLigence and iCELLigence technology, the behavior of cancer cells including proliferation, migration, and invasion in real-time in cell culture can be measured.[65]

Well, the volumes in 16, 36, and 96 well plates in xCELLigence systems lie between 95 and 244 μL, and the resulting data is stored and analyzed using desktop computers and notebooks. RTCA systems are used for the measurement of the alternative encumbrance of adherent cells using interacting microelectrode sensor arrays that are placed at the bottom of electronic (E) plate inserts.[66]

CONCLUSION

Nature has a treatise on the cure for the innumerable sufferings of mankind; continuous exploitation has resulted in the extinction of many important plants and animals. As per the Ayurveda principle, "there is a dynamic balance between one's deeds and fate" therefore, if humans cannot create life, then they have no right to exploit it toward irreparable loss. Exploring cell lines as an alternative to animal experimentation, supplemented by rich Traditional Indian Medicines would not only save the number of experimental animals from painful death but would also be favorable toward the restoration of flora as the number of doses to experimental animals would remarkably decrease. Moreover with the help of newer technologies, receptor binding studies may also be studied in detail. Hence, it is worthwhile to state that the detailed study of Traditional Medicines along with cell line studies in natural product research and diseases is the need of the hour.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

The study was funded by AICTE-MODROBS project grant (F. No. 8024/RID/BOR/MOD-458).

CONFLICTS OF INTEREST

Authors declare no conflicts of interest, financial, or otherwise.

ACKNOWLEDGMENT

The authors would like to thank The Librarian, Central Drugs Research Institute–C.S.I.R.(C.D.R.I.-C.S.I.R), Lucknow, for all the assistance provided during the literature search. The corresponding author is also thankful to the AICTE-MODROBS project grant (F. No. 8024/RID/BOR/MOD-458).

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