



## **ANTICANCER ACTIVITY OF HIBISCUS MUTABILIS**

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### **1. INTRODUCTION**

#### **WHAT IS CANCER?**

Cancer is a disease characterised by uncontrolled multiplication and spread of abnormal forms of the body's own cells his one of the major causes of death in the developed nations, one in three people were diagnosed with cancer during their lifetime and in 2001, about 270,000 new cases were reponed in the UK. Cancer is also responsible for approximately one-quarter of all deaths in the UK with lung and bowel cancer comprising the largest category, closely followed by breast and prostate cancer.

It is estimated that in 2017, the United States alone will have approximately 16,88,780 new cancer diagnoses cases and 6,00,920 cancer death. This uncontrol proliferation of a normal cell which produces genetic instabilities and alterations accumulates within cells and tissues which transform normal cell into a malignant cell. There are several types of cancer in human being among these the lung cancer is reported the top listed in male followed by breast cancer in female. It is a major health burden in both developing and developed countries being treated by medicinal plant as a whole or by their phytochemical very frequently.

Previously, around 10.9 million new cancer cases, 24.6 million persons living with cancer, 6.7 million deaths reported around world each year the Organization data, above 14.1 million new cancer cases and 8.2 million deaths were mentioned globally in the year 2012 and over 70% new cancer cases has been estimated during the next twenty years. Nearly, 80% of the world's population depend on traditional medicines and more than 60% of clinically approved anticancer drugs are derivatives of these medicinal plant. According to literature survey, there are many anticancer drugs clinically approved and are recommended for the cancer treatment The appearance of these abnormal characteristics reflects altered patterns of gene expression in the cancer cells, resulting from genetic mutations.

There are three main approaches to treat established cancer-surgical excision, irradiation and chemotherapy and the relative value of each of these approaches depends on the type of tumour and the stage of its development. In men, the highest percentages of cancer types occur in the prostate, lung and bronchus, colon and rectum, and urinary bladder, respectively. In women, cancer prevalence is highest in the breast, lung and bronchus, colon and rectum, uterine corpus and thyroid, respectively. This data indicates that prostate and breast cancer constitute a major portion of cancer in men and women, respectively. For children, the highest percentage types of cancer disease are blood cancer, and cancers related to the brain and lymph nodes. Cancer occurs by a series of successive mutations in genes so that these mutations change cell functions. Chemical compounds have an obvious role of forming gene mutations and cancer cells. In addition, smoking involves several carcinogenic chemical compounds that lead to lung cancer. Interestingly, environmental chemical substances with carcinogenic properties influence directly or indirectly the cytoplasm and nucleus of cells, and lead to genetic disorders and gene mutations. Viruses, bacteria and radiation rays are other carcinogenesis factors, comprising about 7% of all cancers. In general, cancer disrupts cellular relations and results in the dysfunction of vital genes. This disturbance is affective in the cell cycle, and leads to abnormal proliferation. Proto-oncogenes are responsible for cell division and growth under normal condition, but become oncogenes during genetic mutation, which are most dangerous for cell existence.

In addition, the lack of tumor suppressor genes triggers uncontrolled cells division. Normally, repair genes translate to protein and enzymes that have repairing properties and more than 30 types of detected repair proteins. Removing uracil from DNA bypasses the DNA damage and removes the main DNA lesions induced by ultraviolet light, which are essentially the functions of repair genes to successfully repair DNA . Cancer begins when genetic changes interfere with this orderly process. Cells start to grow uncontrollably. These cells may form a mass called a tumor. A tumor can be cancerous or benign. A cancerous tumor is malignant, meaning it can grow and spread to other parts of the body. A benign tumor means the tumor can grow but will not spread. Some types of cancer do not form a tumor. Types of cancer Doctors divide cancer into types based on where it begins. Four main types of cancer are: Carcinomas. A carcinoma begins in the skin or the tissue that covers the surface of internal organs and glands. Carcinomas usually form solid tumors. They are the most common type of cancer. Examples of carcinomas include prostate cancer, breast cancer, lung cancer, and colorectal cancer. Sarcomas: A sarcoma begins in the tissues that support and connect the body. A sarcoma can develop in fat, muscles, nerves, tendons, joints, blood vessels, lymph vessels, cartilage, or bone. Leukemias: Leukemia is a cancer of the blood. Leukemia begins when healthy blood cells change and grow uncontrollably. The 4 main types of leukemia are acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myeloid

leukemia, and chronic myeloid leukemia. Lymphomas: Lymphoma is a cancer that begins in the lymphatic system. The lymphatic system is a network of vessels and glands that help fight infection. There are 2 main types of lymphomas: Hodgkin lymphoma and non-Hodgkin lymphoma. There are many other types of cancer. Bladder Cancer : Bladder cancer occurs in the tissues of the bladder, which is the organ in the body that holds urine. According to the National Institutes of Health, approximately 45,000 men and 17,000 women per year are diagnosed with the disease. Breast Cancer: It is cancer that develops from breast tissue. Signs of breast cancer may include a lump in the breast, a change in breast shape, dimpling of the skin, fluid coming from the nipple, a newly inverted nipple, or a red or scaly patch of skin. In those with distant spread of the disease, there may be bone pain, swollen lymph nodes, shortness of breath, or yellow skin. Colorectal Cancer: It also known as bowel cancer, colon cancer, or rectal cancer, is the development of cancer from the colon or rectum (parts of the large intestine). Signs and symptoms may include blood in the stool, a change in bowel movements, weight loss, and fatigue Kidney Cancer: It also called renal cancer is a disease in which kidney cells become malignant (cancerous) and grow out of control, forming a tumor. Almost all kidney cancers first appear in the lining of tiny tubes (tubules) in the kidney.

This type of kidney cancer is called renal cell carcinoma. Lung Cancer : It also known as lung carcinoma, since about 98–99% of all lung cancers are carcinomas, is a malignant lung tumor characterized by uncontrolled cell growth in tissues of the lung. Oral and Oropharyngeal Cancer: It is a complex and often relentless malignancy prone to local invasion and dissemination. Despite advances in understanding of the disease and improved therapeutic interventions, it continues to be diagnosed at an advanced stage and the survival rate remains poor. The financial cost of treating OPC may be the highest of all cancers in the United States and survivors often experience major detriments to quality of life. Major risk factors for OPC are tobacco, alcohol, areca nut, and human papillomavirus infection Pancreatic Cancer: It arises when cells in the pancreas, a glandular organ behind the stomach, begin to multiply out of control and form a mass. These cancerous cells have the ability to invade other parts of the body. Prostate Cancer: The prostate is a gland in the male reproductive system that surrounds the urethra just below the bladder. Most prostate cancers are slow growing. Cancerous cells may spread to other areas of the body, particularly the bones and lymph nodes. It may initially cause no symptoms. In later stages, symptoms include pain or difficulty urinating, blood in the urine, or pain in the pelvis or back. Benign prostatic hyperplasia may produce similar symptoms. Other late symptoms include fatigue, due to low levels of red blood cells. Thyroid Cancer: -It develops when cells change or mutate. The abnormal cells begin multiplying in your thyroid and, once there are enough of them, they form a tumor. If it's caught early, thyroid cancer is one of the most treatable forms of cancer. Uterine Cancer: It also known as womb cancer, includes two types of cancer that develop from the tissues of the uterus. Endometrial cancer forms from the lining of the uterus, and uterine sarcoma forms from the muscles or support tissue of the uterus. Endometrial cancer accounts for approximately 90% of all uterine cancers in the United States. Symptoms of endometrial cancer include changes in vaginal bleeding or pain in the pelvis. Symptoms of uterine sarcoma include unusual vaginal bleeding or a mass in the vagina.

### **1.1 The Genesis of Cancer cell:**

A normal cell turns into a cancer cell because of one or more mutations in its DNA which can be acquired or inherited. A good example is breast cancer; women who inherit a single defective copy of either of the tumor suppressor genes BRCA1 and BRCA2 have a significantly increased risk of developing breast cancer. However, carcinogenesis is a complex multistage process, usually involving more than one genetic change as well as other, epigenetic factors (hormonal, cocarcinogen and tumour promoter effects, etc.) that do not themselves produce cancer but which increase the likelihood that the genetic mutation(s) will result eventually result in cancer.

#### **There are two main categories of genetic change that are important:**

The activation of proto-oncogenes to oncogenes. Proto oncogenes are genes that normally control cell division, apoptosis and differentiation, but which can be converted to oncogenes that induce malignant change by viral or carcinogen action. The inactivation of tumour suppressor genes. Normal cells contain genes that have the ability to suppress malignant change termed tumour suppressor genes (anti-oncogene's) and there is now good evidence that mutations of these genes are involved in many different cancers. The loss of function of tumour suppressor genes can be the critical event in carcinogenesis.

About 30 tumor suppressor genes and 100 dominant oncogenes have been identified. The changes that lead to malignancy are a result of point mutations, gene amplification or chromosomal translocation, often caused by viruses or chemical carcinogens.

#### **The pathogenesis of Cancer:**

Cancer cells manifest, to varying degrees, four characteristics that distinguish them from normal cells. These are:

##### **Uncontrolled proliferation:**

Some healthy cells (such as neurons) have little or no capacity to divide and proliferate, whereas others, in the bone marrow and the epithelium of the gastrointestinal tract for example, have the property of continuous rapid division. Some cancer cells multiply slowly (e.g. those in plasma cell tumors) and some much more rapidly (e.g. the cells of Burkitt's lymphoma). It is therefore not generally true that cancer cells proliferate faster than normal cells. The significant issue is that cancer cells have escaped from the mechanisms that normally regulate cell division and tissue growth. Telomeres are specialised structures that cap the ends of chromosomes like the small metal tubes on the end of shoelaces-protecting them from degradation, rearrangement and fusion with other chromosomes. Put simply, DNA polymerase cannot easily duplicate the last few nucleotides at the ends of DNA, and telomeres prevent loss of the 'end' genes. With each round of cell division, a portion of the telomere is eroded, so that eventually it becomes non-functional. At this point, DNA replication ceases and the cell becomes senescent. Rapidly dividing cells, such as stem cells and those of the bone marrow, the germline, and the epithelium of the gastrointestinal tract, express telomerase, an enzyme that maintains and stabilises telomeres. While it is absent from most fully differentiated somatic cells, about 95% of late-stage malignant tumours do express the enzyme, and it is this that may confer 'immortality' on cancer cells. The actual growth of a solid tumour depends absolutely on the development of its own blood supply. Tumours 1-2 mm in

diameter can obtain nutrients by diffusion, but any further expansion requires angiogenesis, the development of new blood vessels. Angiogenesis occurs in response to growth factors produced by the growing tumors.

Inactivation of tumour suppressor genes or transformation of proto-oncogenes into oncogenes can confer autonomy of growth on a cell and thus result in uncontrolled proliferation by producing changes in several cellular systems including:

- Growth factors, their receptors and signaling pathways.
- The cell cycle transducers, for example cyclins, cyclin dependent kinases (cdks) or the cdk inhibitors
- The apoptotic machinery that normally disposes of abnormal cells.
- Telomerase expression.
- Local blood vessels, resulting from tumour-directed angiogenesis.
- Differentiation and loss of function.

The multiplication of normal cells in a tissue begins with division of the undifferentiated stem cells giving rise to daughter cells. These daughter cells eventually differentiate to become the mature cells of the relevant tissue, ready to perform their programmed functions. For example, when fibroblasts mature, they secrete and organize extracellular matrix; mature muscle cells are capable of contraction. One of the main characteristics of cancer cells is that they dedifferentiate to varying degrees. In general, poorly differentiated cancers multiply faster and carry a worse prognosis than well differentiated cancers.

#### **Invasiveness:**

Normal cells are not found outside their site of origin; for example, liver cells are not found in the bladder and pancreatic cells are not found in the testis. This is because, during differentiation and tissue or organ growth, normal cells develop certain spatial relationships with respect to each other. These relationships are maintained by various tissue-specific survival factors that prevent apoptosis. In this way, any cells that escape accidentally lose these survival signals and die.

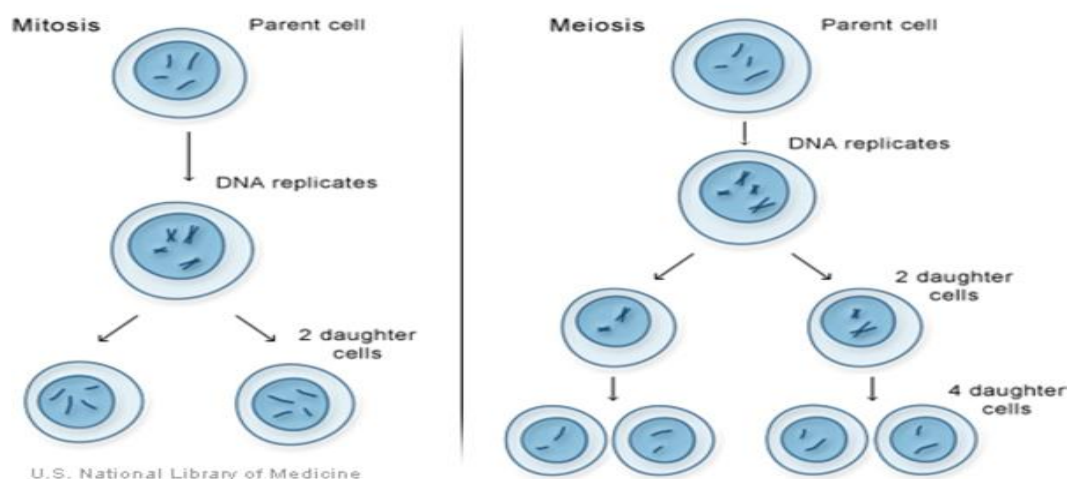
Consequently, although the cells of the normal mucosal epithelium of the rectum proliferate continuously as the lining is shed, they remain as a lining epithelium. A cancer of the rectal mucosa, by comparison, invades other tissues forming the rectum and may even invade the tissues of other pelvic organs. Cancer cells have not only lost, through mutation, the restraints that act on normal cells, but they also secrete enzymes metalloproteinases that break down the extracellular matrix, enabling them to move around.

#### **Metastases:**

Metastases are secondary tumours formed by cells that have been released from the initial or primary tumour and have reached other sites through blood vessels or lymphatics, or as a result of being shed into body cavities. Metastases are the principal cause of mortality and morbidity in most cancers and constitute a major problem for cancer therapy.

Dislodgment or aberrant migration of normal cells would lead to programmed cell death as a result of withdrawal of the necessary antiapoptotic factors. Cancer cells that metastasize have undergone a series of genetic changes that alter their responses to the regulatory factors that control the cellular architecture of normal tissues, enabling them to establish themselves extra territorially. Tumour-induced growth of new blood vessels locally makes metastasis easier and more likely.

Secondary tumours occur more frequently in some tissues than in others. For example metastases of mammary cancers are often found in lung, bone and brain. The reason for this is that breast cancer cells express chemokine receptors such as CXCR4 on their surfaces, and chemokines that recognise these receptors are expressed at high level in these tissues.



### Common signs and symptoms of cancer in both men and women include:

**Pain:** Bone cancer often hurts from the beginning. Some brain tumors cause headaches that last for days and don't get better with treatment. Pain can also be a late sign of cancer, so see a doctor if you don't know why it's happening or it doesn't go away.

Weight loss without trying. Almost half of people who have cancer lose weight. It's often one of the signs that they notice first.

**Fatigue:** If you're tired all the time and rest doesn't help, tell your doctor. Leukemia often wears you out, or you could have blood loss from colon or stomach cancer. Cancer-related weight loss can leave you exhausted, too.

**Fever:** If it's high or lasts more than 3 days, call your doctor. Some blood cancers, like lymphoma, cause a fever for days or even weeks.

Changes in your skin. Have your doctor look at unusual or new moles, bumps, or marks on your body to be sure skin cancer isn't lurking. Your skin can also provide clues to other kinds of cancers. If it's darkened, looks yellow or red, itches, or sprouts more hair, or if you have an unexplained rash, it could be a sign of liver, ovarian, or kidney cancer or lymphoma. Sores that don't heal. Spots that bleed and won't go away are also signs of skin cancer. Oral cancer can start as sores in your mouth. If you smoke, chew tobacco, or drink a lot of alcohol, you're at higher risk. Cough or hoarseness that doesn't go away. A cough is one sign of lung cancer, and hoarseness may mean cancer of your voice box (larynx) or thyroid gland. Unusual bleeding. Cancer can make blood show up where it shouldn't be. Blood in your poop is a symptom of colon or rectal cancer. And tumors along your urinary tract can cause blood in your urine.

**Anemia:** This is when your body doesn't have enough red blood cells, which are made in your bone marrow. Cancers like leukemia, lymphoma, and multiple myeloma can damage your marrow. Tumors that spread there from other places might crowd out regular red blood cells.

### Common Cancer Symptoms in Men:

Trouble peeing: A swollen prostate can make it hard to go, or it may make you have to go a lot. Tell your doctor if you have pain when you pee or blood in your urine. A lump, pain, or ache in your scrotum. These might mean testicular cancer.

### Common Cancer Symptoms in Women:

The types that affect women most are breast, lung, and colorectal cancer. Women can also have cancer of the uterus, endometrium, cervix, vagina, or vulva. Vaginal bleeding or discharge: Get checked out if it happens between periods or after menopause. Endometrial cancer can make you bleed when you don't expect it.

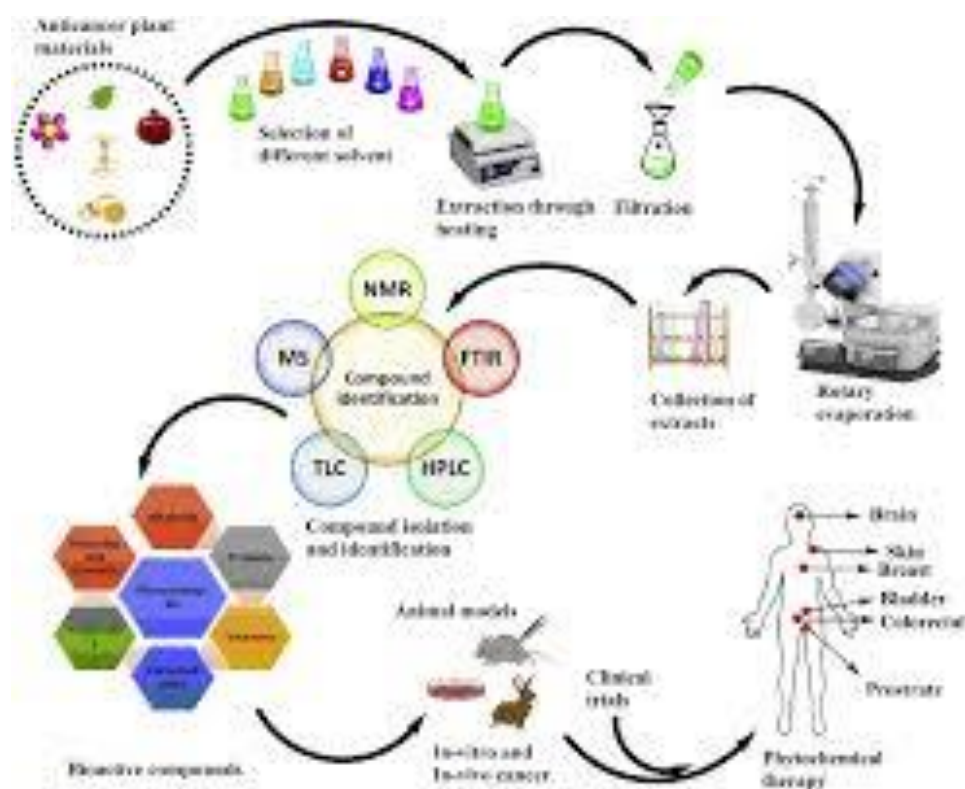
**Changes in appetite:** Ovarian cancer can make you feel full or make it hard to eat. Other cancers can cause indigestion or nausea. Cancer isn't the only illness that changes your appetite. Cancer has been a constant battle globally with a lot of development in cures and preventative therapies. The disease is characterised by cells in the human body continually multiplying with the inability to be controlled or stopped. Consequently, forming tumours of malignant cells with the potential to be metastatic. Current treatments include chemotherapy, radiotherapy and chemically derived drugs. Treatments such as chemotherapy can put patients under a lot of strain and further damage their health. Therefore, there is a focus on using alternative treatments and therapies against cancer.

### 1.2 Need for study of anticancer from plant

For many years herbal medicines have been used and are still used in developing countries as the primary source of medical treatment. Plants have been used in medicine for their natural antiseptic properties. Thus, research has developed into investigating the potential properties and uses of terrestrial plants extracts for the preparation of potential nanomaterial-based drugs for diseases including cancer. Many plant species are already being

used to treat or prevent development of cancer. Multiple researchers have identified species of plants that have demonstrated anticancer properties with a lot of focus on those that have been used in herbal medicine in developing countries. Medicinal plants have been used for thousands of years in folk medicines in Asian and African populations and many plants are consumed for their health benefits in developed nations. According to the World Health Organizations (WHO) some nations still rely on plant-based treatment as their main source of medicine and developing nations are utilising the benefits of naturally sourced compounds for therapeutic purposes [13].

Compounds which have been identified and extracted from terrestrial plants for their anticancer properties include polyphenols, brassinosteroids and taxols. Herbal medicine has become a very safe, non-toxic, and easily available source of cancer-treating compounds. Herbs are believed to neutralize the effects of diseases in a body because of various characteristics they possess. For instance, among the many anticancer medicinal plants, *Phaleria macrocarpa* (local name: Mahkota dewa) and *Fagonia indica* (local name: Dhamasa) have been used traditionally for the anticancer properties of their active ingredients. Metabolites extracted from the plant material are used to induce apoptosis in cancer cells. Gallic acid as the active component was purified from the fruit extract of *P. macrocarpa* and has demonstrated a role in the induction of apoptosis in lung cancer, leukemia, and colon



adenocarcinoma cell lines. It is a polyhydroxy phenolic compound and a natural antioxidant that can be obtained from a variety of natural products i.e., grapes, strawberries, bananas, green tea, and vegetables. It also plays a critical role in preventing malignancy transformation and the development of cancer. Similarly, other compounds such as vinca alkaloids, podophyllotoxin, and camptothecin obtained from various plants are used for the treatment of cancer. Due to lack of effective drugs, cost of chemotherapeutic agents, and the side effects of anticancer drugs, cancer can be a cause of death. Therefore, efforts are still being made to search for effective naturally occurring anticarcinogens that would prevent, slow, or reverse cancer development. Medicinal plants have a special place in the management of cancer. It is estimated that plant-derived compounds in one or the other way constitute more than 50% of anticancer agents. Numerous cancer research studies have been conducted using traditional medicinal plants in an effort to discover new therapeutic agents that lack the toxic side effects associated with the present chemotherapeutic agents. Taking into consideration the above facts, an attempt has been made to evaluate the anticancer, anti-inflammatory, and antioxidant activities of selective medicinal plants used in Indian traditional medicine system. Existing anticancer drugs target rapidly dividing cells. Several cells in our body proliferate quickly under normal circumstances, viz. bone marrow cells, digestive tract cells and hair follicle cells. So these normal cells are also affected by the above mentioned drugs and serious side effects emerge. These side effects include myelosuppression (decreased production of blood cells), mucositis (inflammation of digestive tract lining), hair loss, cardiotoxicity, neurotoxicity and immunosuppression. Additionally, rapid elimination and widespread distribution of the introduced drug to the non-targeted organs by body system requires large dose of the drug which increases above mentioned side effects and also not economic.

### 1.3 Problem in anticancer herbal drug

Although traditional herbal medicines, phytomedicines, medicinal foods, and complementary or alternative have been increasingly used over the past decade in European and North American countries, they seem to have not generated interest or been accepted by mainstream medicine practitioners in western countries, especially in standard care for cancer patients. The key issue considered by many biomedical scientists has been the lack of evidence-based information/guidelines for routine and regulatory application of herbal medicines as “drugs” for use in public health. The sticking points hindering the use of phytomedicines can be attributed to six major issues:

Lack of consistence and reliable sources of authentic medicinal plant

Materials, with respect to species verification and authentication, cultivation using good agricultural practice protocols, and standardized/normalized methods and technology for plant extraction/mixture preparation;

Lack of definitions and routine preparation of the biochemical/biological ingredients and compositions of herbal medicines or the phytochemicals/phytocompounds derived from medicinal plants, with respect to identification of metabolite profiles, index compounds, and putative active compounds or metabolites;

General and specific safety considerations, including tolerable high dosage, minimal effective dosage, and specific usage

Proof of efficacy in treating or assisting specific cancer patients, including lack of results/data from preclinical animal studies, execution of bona fide, and double-blind, placebo-included, statistician-assisted clinical trial studies;

Highly complex “personalized” prescriptions or formulations for the use of some traditional medicines (e.g., in TCM) that may be mystified by a “secret ingredient” in specific formulations; and

The criminal act of supplementation/ “spiking” highly potent western chemical drugs into herbal medicines in counterfeit activities. Without addressing all of the above issues, we cannot meet the challenges of modernizing herbal medicines. Although we have reviewed a spectrum of laboratory, preclinical and clinical studies on potential applications of herbal medicines for cancer patients’ care in an inclusive fashion, a great many of these studies did not follow the stringent requirements, procedures, and protocol for developing western style drug or medicinal foods. Systematic and correlated efforts among researchers of our scientific communities are therefore urgently needed.

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## 2. AIM AND OBJECTIVE

According to literature survey , different pharmacological action had been studied on the different part of Hibiscus Mutabilis by different scientist along with their chemical nature tradition plant is used to antiphlogistic , deparative , febrifuge , stimulant. Flower are used to anyodne , antidotal demulcent ,expectorant and refrige . The plant has reported as folkloric remedy for cancer , so that it was decided to evaluate in vitro anti-cancer activity , of Hibiscus mutabilis on Brine Shrimp.

### Objectives of the study:

1. To Extract Hibiscus mutabilis leaves with solvents
2. To evaluate the in Vitro anticancer activity on brine shrimp.
3. To establish the plant drug and bioactive chemicals as an anticancer medicine

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