Cancer is not just one disease, but a generic term used to encompass a group of more than two hundred diseases sharing common characteristics. Cancers (carcinomas) are characterized by their unregulated growth and spread of cells to other parts of the body\textsuperscript{13} & \textsuperscript{14}.

2.1. Description of Cancer:

Cancer is characterized by alterations in the expression of multiple genes, leading to dysregulation of the normal cellular program for cell division and cell differentiation. This results in an imbalance of cell replication and cell death that favours growth of a tumor cell population. The characteristics that delineate a malignant cancer from a benign tumor are the abilities to invade locally, to spread to regional lymph nodes, and to metastasize to distant organs in the body.

Clinically, cancer appears to possess different phenotypic characteristics. As cancerous growth progresses, genetic drift in the cell population produces cell heterogeneity such as cell antigenicity, invasiveness, as well metastatic potentials.

At the molecular level, all cancers have several things in common, which suggests that the ultimate biochemical lesions leading to malignant transformation and progression can be produced in an unidentical pattern which is due to alterations in gene expression. In general, malignant cancers cause significant morbidity and will be lethal to the host if not treated. Exceptions to this appear to be latent, indolent cancers that may remain clinically undetectable (or \textit{in situ}), allowing the host to have a standard life expectancy.

2.2. Types of cancer:

Human cancers are classified into the following types:

- **Carcinoma**: Malignant tumors derived from epithelial cells. This group represents the most common cancers, including the common forms of breast, prostate, lung and colon cancer.
- **Lymphoma and Leukemia**: Malignant tumors derived from blood and bone marrow cells.
Sarcoma: Malignant tumors derived from connective tissue, or mesenchymal cells.

Mesothelioma: Tumors derived from the mesothelial cells lining the peritoneum and the pleura.

Glioma: Tumors derived from the glial cells, the most common type of brain cell.

Germinoma: Tumors derived from germ cells, normally found in the testicle and ovary.

Choriocarcinoma: Malignant tumors derived from the placenta.

2.3. Predisposing factors of cancer development:

The incidences of cancer can be demonstrated to increase, as society becomes more affluent. Consumption of higher amounts of convenience foods, alcohol and tobacco; Exposure to higher levels of chemicals and pollutants are some of the cancer causing factors. Other factors include past exposure to ionizing radiation and viruses, and a genetic disposition.

2.4. Methods of treatment:

There are a number of treatment options available for cancer. Treatment plans are divided depending on the type of cancer, its site of occurrence and the developmental stage. The three mainlines of cancer treatment are:

- Surgery,
- Radiotherapy and
- Chemotherapy.

2.4.1. Surgery:

Surgical removal of tumour is one of the important main lines of treatment. Tissues adjacent to the cancer cells were also removed into which cancer might have spread. Surgery is the most effective form of treatment in the management of cancer.
For many cancers, especially for early skin cancers and small cancers on lips or in the mouth, surgery offers a relatively simple, straightforward, quick and effective method of treatment. A complete cure can be expected without any residual problem.

2.4.2. Radiotherapy:

Radiotherapy is the second oldest effective form of treating cancer. Treatment depends upon the sensitivity of dividing cells which is being destroyed by X-rays or gamma rays emitted from a radioactive source. This in turn results in eradicating cancer cells and causing minimal damage to surrounding non-cancerous cells. Radiotherapy method of treatment is at present improved with advancements in equipments and computer technologies.

2.4.3. Chemotherapy:

Chemotherapy is the method of treatment which involves the usage of chemical agents. The following agents are effectively used in chemotherapy:

- Alkylating Agents
- Cytotoxic Antibiotics And Amsacrine
- Anti-Metabolites
- Vinca Alkaloids And Etoposide
- Platinum Compounds
- Taxanes
- Camptothecins
- Non-Cytotoxic Cancer Agents
- Tyrosine Kinase Inhibitors
- Proteosome Inhibitors
- Monoclonal Antibodies
2.4.4. Side Effects of Chemotherapy:

The risk of using cytotoxic anti-cancer drugs is that, normal tissues may also get damaged. Expert supervision should ensure maximum damage to cancer cells and minimum damage to normal cells and normal body tissues. The common side effects include:

- Fatigue
- Skin Problems
- Hair Loss
- Loss of Appetite
- Digestive Tract Problems
- Abnormality on Sexual and Reproductive Functions
- Lymphodema.

2.5. Molecular mechanism of carcinogenesis:

Cancers develop through a stepwise process with the acquisition of activating mutations in dominant growth enhancing “oncogenes” and inactivating recessive mutations in growth inhibitory TSG. In addition, epigenetic abnormalities in the expression of these genes also play an important role in carcinogenesis.

Detailed analysis of the diverse functions of the known oncogenes and TSG shows that they code for components of the signal transduction cascade, i.e. growth factors, growth factor receptors, adapter molecules, protein kinases, G-proteins, nuclear transcription factors, molecules that repair DNA, regulate the cell cycle and the various check points mediating apoptosis, metastasis and invasion. This catalogue of genes manifest six essential alterations in physiology that collectively dictate malignant growth, i.e. growth signal autonomy, insensitivity to growth-inhibitory signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, tissue invasion and metastasis. These six capabilities are shared in common by most and perhaps all types of human cancers.\(^{16}\)
2.5.1. Growth signal autonomy:

The complex mechanisms which lead to *growth signal autonomy* arise from alterations in components of the downstream signal transduction pathways initiated by ligand activated growth factor receptors and integrins which regulate the cell-cycle. During mitogenic stimulation, growth factors bind to their receptors and promote their dimerization and autophosphorylation. This leads to the activation of SH2 domain containing proteins such as, PLC\(_g\), PI3K, the oncoproteins Ras and Src and, in turn, the MAP kinase cascade. End products of these cascades, MAPK, p38, and JNK are translocated to the nucleus where they phosphorylate and activate many substrates including transcriptional factors such as Jun, Ets1, Ets2, Tcf, etc. This causes activation of other transcription factors.

Similar reactions are also observed on binding of integrins with ECM proteins which promote the autophosphorylation of FAK. This result in the binding of FAK to the SH2 domain of the Src proto-oncoprotein followed by recruitment of the adapter Grb2 protein, and activation of Ras and MAP kinase cascades. The major consequence of the MAP kinase activated transcription factors is the increase in expression of the oncoprotein cyclin D1 and Myc, which increase the activity of cyclin-dependent kinases operating in G1 phase (cyclin D–Cdk4 and cyclin E– Cdk2). These phosphorylate the tumour suppressor protein pRb. Phosphorylation of pRb and its binding to a number of oncoproteins induces release and activation of transcription complexes E2F/DP, which increase expression of genes whose products are necessary for passage of the S phase\(^{17}\), leading to continuous cell multiplication.

2.5.2. Insensitivity to antigrowth signals:

*Insensitivity to antigrowth signals* and uncontrolled proliferation involves the Cdk–Rb–E2f signalling pathway. This is controlled not only by pRb, but also by many other suppressor proteins, some of which are inhibitors of Cdns, promoting arrest of the cell cycle at the G0/G1 phase, so that the S phase does not begin in response to various signals. These proteins are p15\(^{INK4b}\), p16\(^{INK4a}\), p21\(^{WAF1/CIP1}\), p27\(^{KIP1}\), and p57\(^{KIP2}\).\(^{18}\) Proteins p27\(^{KIP1}\) as well as p15\(^{INK4b}\) are activated by the inhibitory signal transduction
cascade induced by TGFβ binding to its receptors. The protein p21WAF1 is one of the main targets for the transactivating effect of p53 and consequently for suppressors involved in the stability/activity of p53, i.e. p19ARF, ATM, WTI or its transcriptional activity (BRCA1 and p33ING1). The antigrowth circuit converging on Rb and the cell division cycle is disrupted in a majority of human cancers thereby defining the concept of tumour suppressor loss in cancer.

To conclude, most known proto-oncogenes and TSG somehow regulate activity of cyclin-dependent kinases which are responsible for entrance to the S-phase. Impairment in the signalling pathways-regulating-Cdk 2,4,6-pRb-E2F/DP is therefore a necessary precondition for the appearance of constantly proliferating neoplastic cells.

It is apparent from the above delineations that the current paradigm in carcinogenesis is associated with activation of oncogenes and decreased expression of TSG. In several types of human cancers there is an increase in the expression of the products of the TSG, p27, p21, p16 or Rb.

2.5.3. Resistance to programmed cell death:

The ability of the tumour cells to expand in number is determined not only by the rate of cell proliferation but also by resistance to programmed cell death. At least two different pathways seem to promote apoptosis by activation of aspartate proteases—caspases which act on key substrates leading to cell death. Binding of death factors, Fas ligand and TNFα to their specific receptors generates caspases 3, 6 and 7, which are pivotal for apoptosis. An alternative mechanism involves the generation of caspases 3, 6 and 7 via caspase 9 which is activated via a pathway involving the TS p53. Activation of p53 by several apoptotic signals such as DNA damage, activation of oncogenes, survival factor insufficiency or hypoxia, loss of cell contacts with other cells or the ECM leads to the regulation of the Bcl2 family of proteins, in particular increase in expression of BAX gene and repression of the BCL2 gene.
The Bax protein promotes release of cytochrome c and/or AIF (apoptosis-inducing factor) from the mitochondria. The Bcl₂ and Bclx proteins inhibit this release. Bax in turn can bind to Bcl₂ and Bclx and negate their effects. Cytochrome c and the protease AIF are instrumental in activating caspase 9 which in turn generates caspases 3, 6 and 7 leading to apoptosis (Fig 1). Resistance to apoptosis can be acquired by cancer cells through mutation in the tumour-suppressor p53 gene, upregulation of Bcl₂ oncogene via chromosomal translocations as in follicular lymphoma²⁴ by other strategies. Apoptosis is also initiated by insufficient survival factors such as IGF-1/IGF-2, and IL-3 and their receptors²⁵. Alterations in the PI-3 kinase-AKT/PKB pathway, may be due to transmission of antiapoptotic survival signals²⁶ or by loss of the PTEN, TSG which attenuates the AKT survival signal and also lead to cancer cells evading apoptosis²⁷.

2.5.4. Limitless replicative potential:

Cell proliferation can be regulated arresting the cell cycle which is a precondition for differentiation. The process of ageing and immortalization is as important as the complex formation that regulates life and death. Cells in culture have a finite replicative potential. After a finite number of doublings they stop growing—a process termed senescence. This process can be circumvented by disabling their pRb and p53 proteins, which permit the cells to continue doubling till they reach a crisis state. This is characterized by massive cell death, karyotypic disarray associated with end-to-end fusion of chromosomes and the occasional emergence of a variant cell that has acquired the ability to multiply without limit, the trait termed immortalization. Most types of tumour cells are immortalized.

The immortality of the cancer cells is being identified in the ends of the chromosomes, the telomeres which are composed of several thousand repeats of a short 6 bp sequence TTAGGG element. The normal shortening process seen in the telomeres during successive cellular divisions that eventually causes ageing and cell death. This process is reverted by the stabilization of the telomere through the action of a complex ribonucleoprotein enzyme known as telomerase²⁸. This enzyme is found in embryonic
and germinal cells, but in undetectable levels in normal eukaryotic cells, except in tissues which are turning over. Virtually all types of malignant cells maintain their telomeres by upregulating expression of the telomerase enzyme. The activity of this enzyme is controlled by the Myc oncoprotein, which increases the transcription of the gene encoding the TERT subunit and determines telomerase activity\textsuperscript{29}.

2.5.5. Sustained angiogenesis:

Nutrients and oxygen required for tumour growth are supplied by the formation of new capillary networks from endothelial cells lining small venules. This process which is known as neoangiogenesis, is regulated by the termination of secretion of inhibitors and growth factors, such as VEGF, FGF, EGF and TGF\alpha, which are required for the proliferation and migration of endothelial cells.

Increase in growth factors is accompanied by increase in secretion and/or activity of proteases, leading to the proteolysis of the extra-cellular matrix and endotheliocyte invasion of the neoplastic tissue. The TS p53 activates production of the inhibitor thrombospondin and suppresses the transcription of VEGF gene\textsuperscript{30}.

Inactivation of p53 therefore plays a key role in the formation of the angiogenic phenotype of neoplastic cells. The Ras oncogene family induces activation of transcriptional complex AP-1 and increases VEGF secretion and production of MMP9 and 1\textsuperscript{31}. The oncogene proteins Myc and Vhl are also reported to play a role in angiogenesis\textsuperscript{32 & 33}. 

PHARMACOGNOSTIC STANDARDIZATION AND BIOCHEMICAL EVALUATION OF TRADITIONAL ANTICANCER DRUG SOURCES - \textit{ARTEMISIA SPP.}
Growth control and the cell cycle, the heart of cancer, are influenced by many types of signals, and the external inputs become integrated as the cell makes its decision about whether to divide or to continue dividing. Developmental pathways give cells their identity, and along with that identity often comes a commitment to proliferate or not. Genes known to be mutated in cancer cells are highlighted in red. Less firmly established pathways are shown with dashed lines. 

PHARMACOGNOSTIC STANDARDIZATION AND BIOCHEMICAL EVALUATION OF TRADITIONAL ANTICANCER DRUG SOURCES - ARTEMISIA SPP.
2.5.6. Tissue invasion and metastasis:

As the cancer progresses, there is further loss of control whereby the *tumour cells escape* from the primary foci and move to distant sites where they lodge and form new foci. One of the better studied cell surface molecules is E-cadherin, which is also a product of a TSG, mediating cell to cell interactions resulting in transmission of antigrowth and other signals via *b*-catenin and terminating in the activation of the Lef/Tcf transcription factors. E-cadherin function is lost in a majority of epithelial cancers, by mutational inactivation of the E-cadherin or *b*-catenin genes and transcriptional repression or proteolysis of the extracellular cadherin domain\(^35\).

The proteins p53, Ras and Src are of major importance because changes in their activity cause simultaneous appearance of a few components of the metastatic phenotype and genetic instability that promotes the appearance of additional alterations required for metastasis\(^36\).

The following illustration ([Fig 2](#)) explains the gradual accumulation of mutations in a cell on its way to become a full blown cancerous cell, with colon cancer as a model.
Suppressor gene in a single epithelial cell causes the cell to divide forming a mass of localized benign tumour cells, or polyp. Subsequent mutations leading to expression of a constitutively active Ras protein and loss of two tumour suppressor genes – an unidentified gene in the vicinity of DCC and p53 – generate a malignant cell carrying all four mutations. This cell continues to divide, and the progeny invade the basal lamina that surrounds the tissue. Some tumour cells spread into blood vessels that will distribute them to other sites in the body. Additional mutations permit the tumour cells to exit from the blood vessels and proliferate at distant sites, cancer development.
Discovery and functional characterization of genes in these regions and the order, if any in which they occur, should lead to improved understanding of the process of carcinogenesis and progression. Several of the cancer genes and their products are already proving to be useful as ‘tumour markers’ and some of them as targets for cancer therapy\textsuperscript{38}.

To summarize, the molecular mechanism of carcinogenesis is associated with activation of oncogenes and decreased expression of Tumor Suppressor Gene. The anticancer plant material to be selected for the study possibly should contribute to one or more of the following mechanisms of carcinogenesis:

- Alterations in components of the downstream signal transduction pathways which leads to the activation of MAP kinase cascade.
- Impairment in the signalling pathways-regulating-Cdk 2,4,6-pRb-E2F/DP which leads to constitutive replication of cells.
- Inhibition of the Bax protein by Bcl\(_2\) and Bclx proteins. Alterations in the PI-3 kinase-AKT/PKB pathway, which transmit antiapoptotic survival signals and lead to cancer cells evading apoptosis.
- Suppression of pRb and p53 proteins and upregulated expression of the telomerase enzyme leading to alterations in expression of Myc oncoprotein which are the causes for limitless replicative potential.
- Inhibition of inhibitor thrombospondin and activation of transcription of \textit{VEGF} gene that is responsible for neoangiogenesis.
- Activation of \textit{Ras}, Myc and Vhl oncogene family that are also reported to play a key role in angiogenesis.
- Loss in the function of E-cadherin and \(b\)-catenin genes and transcriptional repression or proteolysis of the extracellular cadherin domain leading to tissue invasion and metastasis by causing change in the activities of the proteins p53, Ras and Src.