INTRODUCTION
1.1 CANCER
Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells\(^1\). It is caused by both external factors (e.g., tobacco, infectious organisms, chemicals and radiation) and internal factors (inherited mutations, hormones, immune conditions and mutations that occur from metabolism). These causal factors may act together or in sequence to initiate or promote carcinogenesis. Cancer involves dynamic changes in the genome\(^2\). By now, several mutations have been discovered that produce oncogenes with dominant gain of function and tumour suppressor genes with recessive loss of function\(^3\). Tumourigenesis in humans is a multistep process and these steps reflect the genetic alterations that drive the progressive transformation of normal human cells into highly malignant ones.

![Diagram of normal cells developing into cancer cells](image)

**Figure no. 1: Development of carcinomatous lesions**

Many types of cancers are diagnosed in the human population with an age-dependent incidence\(^4\). Colorectal cancers are amongst such cancers. Pathological analysis of a number of organ sites reveal lesions that may represent the intermediate steps in a process through which cells evolve progressively from normalcy via a series of premalignant states into invasive cancers\(^5\).
It is now known that genomes of tumour cells are invariably altered at multiple sites, having suffered disruptions through lesion, as subtle as point mutations and as obvious as changes in chromosome complement. Thus, tumour development proceeds via a process in which a succession of genetic changes; each conferring one or another type of growth advantage, leads to a progressive conversion of normal human cells into cancer cells.

Moreover, cancer cells have defects in regulatory circuits that govern cell proliferation and homeostasis. The vast catalogue of cancer cell genotypes may be considered as a manifestation of six essential alterations in cell physiology that collectively dictate malignant growth:

- Self-sufficiency in growth signals
- Insensitivity to growth-inhibitory (antigrowth) signals
- Evasion of programmed cell-death (apoptosis)
- Limitless replicative potential
- Sustained angiogenesis
- Tissue invasion and metastasis

Each of these physiological changes acquired during tumour development represent the successful breaching of an anticancer defence mechanism hardwired into cells and tissue.

Figure no.2: Acquired capabilities of cancer
1.1.1 ACQUIRED CAPABILITIES: SELF-SUFFICIENCY IN GROWTH SIGNALS
Normal cells require mitogenic growth signals before they can move from a quiescent stage into an active proliferative stage. These signals are transmitted into the cells by transmembrane receptors that bind distinctive classes of signaling molecules such as:

- Diffusible growth factors
- Extracellular matrix components
- Cell-to-cell adhesion/interaction molecules

Normal cells cannot proliferate in absence of these stimulatory signals. Many of the oncogenes act by mimicking the normal growth signaling in one way or another. The tumour cells generate many of their own growth signals, thereby reducing their dependence on stimulation from their normal tissue microenvironment. This freedom of dependence from the exogenously derived signals disrupts a critically important homeostatic mechanism that normally operates to ensure proper behaviour of various cell types within a tissue.

1.1.2 ACQUIRED CAPABILITIES: INSENSITIVITY TO ANTIGROWTH SIGNALS
Within a normal tissue, multiple anti-proliferative signals operate to maintain cellular quiescence and tissue homeostasis. These signals include both soluble growth inhibitors and immobilized inhibitors embedded in the extracellular matrix and on the surfaces of nearby cells. These growth-inhibitory signals, like their positive counterparts are received by transmembrane cell surface receptors coupled to intracellular signaling circuits. Antigrowth signals can block cellular proliferation by two pathways:

- Cells may be forced out of active proliferative cycle into the quiescent G0 state from which they reemerge on some future occasion when extracellular signals permit.
- Cells may be forced to permanently abandon their proliferative potential by being induced to enter post mitotic states and undergo specific differentiation. Incipient cancer cells have to evade these anti proliferative signals to prosper.
1.1.3 ACQUIRED CAPABILITIES: EVADING APOPTOSIS

The ability of tumour cell population to expand in number is determined not only by increase in cell proliferation but also by decrease in cell destruction—apoptosis or programmed cell death. Acquired resistance towards apoptosis is the hallmark of most cancers. The apoptotic programme is present in its latent form in virtually all cells of the body. Once triggered by various physiological signals, this programme unfolds in a precisely monitored series of steps. Cellular membranes are disrupted, the cytoplasmic and nuclear skeletons are broken down, the cytosol is extruded, the chromosomes are degraded, and the nucleus is fragmented, all in a span of 30-120 min. In the end, the shriveled cell corpse is engulfed by the nearby cells and disappears, typically within 24h\(^{10}\).

The apoptotic machinery can be broadly divided into two classes of components:

- Sensors and
- Effectors

The sensors are responsible for monitoring the extracellular and intracellular environment to detect any signals which dictate whether the cell should live or die. These signals regulate the second class of components, which function as effectors of apoptotic death\(^{11}\).

Intracellular sensors monitor the cell’s well-being and activate the death pathway in response to detecting abnormalities, including DNA damage, signaling imbalance provoked by oncogene action, survival factor insufficiency or hypoxia\(^{12}\). Now, the life of the cell is maintained by cell-matrix and cell-cell adherence based survival signals whose abrogation elicits apoptosis\(^{13, 14}\). Both soluble and immobilized apoptotic regulatory signals likely reflect the needs of the tissue to maintain their constituent cells in appropriate configurations.

Many of the signals that elicit the apoptotic response converge on the mitochondria. The mitochondria, in response to such proapoptotic signals release cytochrome \(c\), a potent catalyst of apoptosis\(^{15}\). Members of the Bcl2 family of proteins, whose members have either proapoptotic (Bax, Bak, Bid, Bim) or antiapoptotic (Bcl-2, Bcl-xL, Bcl-W) function, act in part by governing the mitochondrial death signaling through cytochrome \(c\) release. The p53 tumour suppressor protein can elicit apoptosis by upregulating expression of proapoptotic Bax in response to sensing DNA damage; Bax in turn, stimulates the mitochondria to release cytochrome \(c\).
The ultimate effectors of apoptosis include an array of intracellular proteases termed caspases\textsuperscript{16}. Two “gatekeeper” caspases, -8 and -9, are activated by death receptors such as Fas ligand (CD95L- Cluster of differentiation) or by the cytochrome \( c \) released by the mitochondria, respectively. These proximal caspases trigger the activation of a dozen or more effector caspases that execute the death programme, through selective destruction of sub-cellular structures and organelles, and of the genome.

Resistance to apoptosis can be acquired by cancer cells through a variety of strategies—the most commonly occurring being the mutation of proapoptotic regulator p53—the tumour suppressor gene. The resulting functional inactivation of its product, the p53 protein is seen in more than 50% of the cancers\textsuperscript{17}. The latter is a key component of the DNA damage sensor that can induce the apoptotic effector cascade. Signals evoked through other abnormalities such as hypoxia and oncogene hyperexpression are also funneled in part via p53 to the apoptotic machinery; these too are impaired at eliciting apoptosis when p53 function is lost\textsuperscript{18}. Virtually all cancer cells harbour alterations that enable evasion of apoptosis.

It is known that in the apoptotic signaling circuitry, most regulatory and effector components are present in the redundant form. This redundancy holds important implications for the development of novel forms of antitumour therapy, since tumour cells that have lost proapoptotic components are likely to retain other similar ones. It is hoped that the apoptotic pathways may still be functional in specific types of cancer cells and new drug entities may enable cross-talk between still intact components of parallel apoptotic signaling pathways in tumour cells, resulting in restoration of apoptotic defence mechanism, with substantial therapeutic benefit.

1.1.4 ACQUIRED CAPABILITIES: LIMITLESS REPLICATIVE POTENTIAL

Three acquired capabilities—growth signal autonomy, insensitivity to antigrowth signals and resistance to apoptosis—all lead to an uncoupling of a cell’s growth programme from signals in its environment. The resulting deregulated proliferation programme should suffice to enable generation of vast populations that constitute macroscopic tumours\textsuperscript{19}.
1.1.5 ACQUIRED CAPABILITIES: SUSTAINED ANGIOGENESIS

The oxygen and nutrients supplied by the vasculature are crucial for cell function and survival, obligating virtually all cells in a tissue to reside within 100 µm of a capillary blood vessel. Once a tissue is formed, the growth of the new blood vessels— the process of angiogenesis— is transitory and carefully regulated. Because of this dependence on nearby capillaries, it would seem plausible that proliferating cells within a tissue would have an intrinsic ability to encourage blood vessel growth. However, it is seen that the cells within aberrant proliferative lesions initially lack angiogenesis ability, curtailing their capability for expansion. In order to progress to a larger size, incipient neoplasias must develop angiogenic ability\(^{20-22}\). Counterbalancing positive and negative signals encourage or block angiogenesis. The angiogenesis-initiating signals are exemplified by vascular endothelial growth factor (VEGF) and fibroblast growth factors (FGF). There are more than two dozen angiogenic inducer factors and a similar number of endogenous inhibitor proteins known today (about 27 endogenous inhibitors are known such as Arresten, Interferons, Troponin I)\(^{23}\).

The mechanisms underlying shifts in the balance between angiogenic regulators remain incompletely understood. For example, an inhibitor- thrombospondin-1 is known to be positively regulated by p53; consequently, loss of p53 function causes thrombospondin-1 levels to fall, liberating endothelial cells from its inhibitory effects\(^ {24}\). The VEGF gene is also under complex transcriptional control\(^ {25, 26}\). As is apparent, inhibition of tumour angiogenesis offers a unique therapeutic target. However, different types of tumour cells use distinct molecular strategies to activate the angiogenic switch. Hence, a single agent may not be able to treat all tumour types.

1.1.6 ACQUIRED CAPABILITIES: TISSUE INVASION AND METASTASIS

Sooner or later, during the development of most types of human cancers, primary tumour masses spawn pioneer cells that move out, invade adjacent tissues and thence travel to distant sites where they may succeed in founding new colonies. These distant settlements of tumour masses— metastases— are the cause of 90% human cancer deaths\(^ {27}\).

The capability of invasion and metastasis enables cancer cells to escape the primary tumour mass and colonize a new terrain elsewhere in the body where at least initially
nutrients and space are not limiting. Like the formation of primary tumour mass, successful invasion and metastasis depend on all of the other five hallmark capabilities. Invasion and metastasis are exceedingly complex processes and at mechanistic levels, they are closely related. This justifies their association with one another. The acquired capability for invasion and metastasis represents the last great frontier for exploratory cancer research and the challenge will be to apply the new molecular insights about tissue invasiveness and metastasis to the development of effective therapeutic strategies.

The above six capabilities during the course of tumour progression are acquired, directly or indirectly, through changes in genomes of cancer cells. Mutations of specific genes are an inefficient process, reflecting the unceasing maintenance of genomic integrity by a complex array of DNA monitoring and repair enzymes. These strive to ensure that the DNA sequence information remains pristine and that mutations are rare events; indeed so rare that the multiple mutations known to be present in tumour cell genomes are highly unlikely to occur within a human life span. Yet cancers do appear at substantial frequency. This implies that the genomes of tumour cells must acquire increased mutability in order for the process of tumour progression to reach completion in several decades time\textsuperscript{28}. Malfunction of specific components of these “caretaker” systems, e.g. of p53 may explain this increased tendency towards mutability and genomic instability\textsuperscript{29} which leads to generation of mutant cells with selective advantages.

1.2 THE LARGE INTESTINE (COLON)

1.2.1 ANATOMY OF THE COLON

The large intestine (colon) is the terminal end of the gastrointestinal (GI) tract and its functions are to complete the process of absorption, production of certain vitamins and formation and expulsion of the fecal matter from the body. The large intestine, which is about 1.5 m long and 6.5 cm in diameter, extends from the ileum to the anus\textsuperscript{30}. Structurally, the four major regions of the large intestine are cecum, colon, rectum and anal canal.
Introduction

The opening from the ileum into the large intestine is guarded by a fold of the mucous membrane called the ileocecal sphincter, which allows material from the small intestine to pass into the large intestine. Hanging inferior to the ileocecal valve is the cecum, a small pouch about 6 cm long. Attached to the cecum is a twisted, coiled tube, measuring about 8 cm in length, called the vermiform appendix. The open end of the cecum merges with the colon, which is divided into the ascending, transverse, descending and sigmoid portions.

Both the ascending and descending colon are retroperitoneal; the transverse and the sigmoid are not. The ascending colon ascends on the right side of the abdomen, reaches the inferior surface of the liver, and turns abruptly to the left to form the right colic (hepatic) flexure. The colon continues across the abdomen to the left side as the transverse colon. It curves beneath the inferior end of the spleen on the left side as the left colic (splenic) flexure and passes inferiorly to the level of the iliac crest as the descending colon. The sigmoid colon begins near the iliac crest, projects medially to the midline and terminates as the rectum, the last 2 cm of the GI tract. The terminal 2-3cm of the rectum is called the anal canal.

Figure no. 3: Anatomy of the large intestine

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1.2.2 HISTOLOGY OF THE COLON
The wall of the GI tract from the lower esophagus to the anal canal has the same basic, four-layered arrangement of tissues. The four layers of the tract, as seen in figure no. 4, from deep to superficial, are the mucosa, submucosa, muscularis and serosa.

1.2.2.1 MUCOSA
The mucosa, or inner lining of the GI tract, is a mucous membrane. It is composed of:
(1) a layer of epithelium that is in direct contact with the contents of the GI tract,
(2) a layer of connective tissue called the lamina propria, and
(3) a thin layer of smooth muscle called the muscularis mucosae.

1. The epithelium contains mostly absorptive and goblet cells. The absorptive cells function primarily in water absorption; the goblet (exocrine) cells secrete mucus that lubricates the passage of the colonic contents. The rate of renewal of GI tract epithelial cells is rapid: every 5 to 7 days they slough off and are replaced by new cells. Located among the epithelial cells are several types of enteroendocrine cells that secrete hormones.

2. The lamina propria is an areolar connective tissue which contains many blood and lymphatic vessels. This layer supports the epithelium and binds it to the muscularis mucosae. The lamina propria also contains the majority of the cells of the mucosa-associated lymphatic tissue (MALT). These prominent lymphatic nodules contain immune system cells that protect against disease.

3. A thin layer of smooth muscle fibers called the muscularis mucosae throws the mucous membrane of the small intestine into many small folds, which increase the surface area for digestion and absorption.

1.2.2.2 SUBMUCOSA
The submucosa consists of areolar connective tissue that binds the mucosa to the muscularis. It contains many blood and lymphatic vessels that receive absorbed food material. Also located in the submucosa is an extensive network of neurons known as the submucosal plexus. The submucosa may also contain glands and lymphatic tissue.
1.2.2.3 MUSCULARIS
Throughout the GI tract, the muscularis consists of smooth muscles that are generally found in two sheets: an inner sheet of circular fibers and an outer sheet of longitudinal fibers. Involuntary contractions of the smooth muscles help to break down food, mix it with digestive secretions and propel it along the tract. Between the layers of the muscularis is a second plexus of neurons - the myenteric plexus.

1.2.2.4 SEROSA
Those portions of the GI tract that are suspended in the abdominopelvic cavity have a superficial layer called the serosa. This is a serous membrane composed of areolar connective tissue and simple squamous epithelium (mesothelium). The serosa is also called the visceral peritoneum because it forms a portion of the peritoneum.
1.3 INTRODUCTION TO COLORECTAL CANCERS (CRC)

Histological observations have led to the concept that most colorectal cancers develop from normal epithelium through sequentially worsening degrees of adenomatous dysplasia i.e. these cancers develop as a result of stepwise progression wherein tumourigenesis initiates in the normal mucosa with a generalized disorder of cell replication and with the appearance of clusters of enlarged (aberrant) crypts showing proliferative, biochemical and biomolecular abnormalities\(^1\), \(^2\). Adenomatous polyps thus, can be defined as well demarcated masses of epithelial dysplasia with uncontrolled crypt cell division. An adenoma can be considered malignant when neoplastic cells pass through muscularis mucosae and infiltrate the submucosa\(^3\). It may be pedunculated when it possesses a stalk; sessile adenomas rise above the background mucosa without any stalk. Small adenomas (< 1 cm) are known as “diminutive”.

Figure no. 5: Three dimensional view of layers of the large intestine
The genetic pathway model for the pathogenesis of sporadic colorectal cancer proposed by Fearon and Vogelstein is based on this concept of an adenoma to carcinoma sequence\textsuperscript{34}. Although the total accumulation of mutations is the principal factor, the model proposed that the causative mutations in tumour suppressor genes and oncogenes occur in a specific order in most colorectal cancers (specifically, adenomatous polyposis coli (APC) gene mutations, global hypomethylation, \textit{k-ras} mutations, deleted in colon cancer (DCC) gene mutations and finally mutations in the \textit{p53} gene. Inactivation of the \textit{APC}\textsuperscript{35-40} gene on chromosome 5 is one of the earliest steps that can result in adenoma formation. Other important genetic changes are mutation and loss of heterozygosity (LOH) of the \textit{p53} gene, LOH of chromosomes 8p, 17p, 18q and 22q and mutation of \textit{k-ras}\textsuperscript{41-44}. The progressive loss of many tumour- suppressor loci drives tumourigenecity and depends on the acquisition of a state of ‘chromosomal instability’\textsuperscript{45}. 

\textbf{Figure no. 6: Developmental stages of an adenomatous polyp.}
Although carcinomas usually originate from pre-existing adenomas, it does not imply that all polyps undergo malignant changes, and does not exclude “de novo” carcinogenesis. Besides adenomas, other types of polypoid lesions include hyperplastic polyps (showing elongated crypts often with cystic dilatation), serrated adenomas (with a serrated glandular pattern), flat adenomas (flat lesions which may possess malignant potential), harmartomatous polyps (which show a complex branching pattern of smooth muscle supporting normal lamina propria and glands) and inflammatory polyps.

The disease may be diagnosed in patients with a familial history of the disease (between 5-20% of the incidences of colorectal cancers fall in this category\textsuperscript{46-49}) or in patients with sporadic adenomatous colorectal polyps or extensive ulcerative colitis\textsuperscript{50,51}. Individuals with certain known single-gene disorders are at an increased risk of developing rectal cancer. Single-gene disorders related to known syndromes account for about 10 to 15% of colorectal cancers.
The hereditary colorectal cancer syndromes and some genes that are involved include\textsuperscript{38, 52-54}:

A. Nonpolyposis disorders
- Hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome\textsuperscript{55-62}: mismatch repair (MMR) genes.

B. Polyposis disorders
- Familial adenomatous polyposis (FAP) \textsuperscript{63, 64}: Adenomatous polyposis coli\textsuperscript{65} (APC) gene.
- Turcot syndrome\textsuperscript{66-68}: APC gene; Mismatch repair (MMR) genes.
- Attenuated familial adenomatous polyposis (AFAP) \textsuperscript{69, 70}: APC gene.
- Hyperplastic polyposis syndrome\textsuperscript{71, 72}: v-raf murine sarcoma viral oncogene homolog B1 (BRAF) \textsuperscript{73, 74} and V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS)\textsuperscript{75} genes.

C. Hamartomatous disorders
- Peutz-Jeghers syndrome (PJS)\textsuperscript{76, 77}: Serine/threonine kinase 11 gene (STK11); also known as liver kinase B1 (LKB1)\textsuperscript{78} gene.
- Juvenile polyposis syndrome (JJP)\textsuperscript{79}: (Homologs of both the drosophila protein mothers against decapentaplegic (MAD) and the C. elegans protein SMA) SMAD4/Deleted in pancreatic cancer, locus 4 (DPC4)\textsuperscript{80} and Bone morphogenetic protein receptor type-1A BMPRIA genes.
- Cowden syndrome (CD): Phosphatase and tensin homolog (PTEN) gene\textsuperscript{81}.
- Ruvalcaba–Myhre–Smith syndrome\textsuperscript{82}: PTEN gene.
- Hereditary mixed polyposis syndrome.
The commonest hereditary syndromes are familial adenomatous polyposis and heredity non-polyposis colon cancer. Attenuated familial adenomatous polyposis, juvenile polyposis syndrome and Peutz-Jeghers syndrome are rarer, mendelian causes of colorectal cancer. In familial adenomatous polyposis (a mendelian dominant disorder with almost complete penetrance) there is a germline mutation in the tumour suppressor gene for \textit{APC} on chromosome 5. Heredity non-polyposis colon cancer also shows dominant inheritance, and cancers develop mainly in the proximal colon. Patients with heredity non-polyposis colon cancer show germline mutations in DNA mismatch repair enzymes (which normally remove mis-incorporated single or multiple nucleotide bases as a result of random errors during recombination or replications). In addition to the well recognized syndromes described above, clusters of colorectal cancer occur in families much more often than would be expected by chance. Postulated reasons for this increased risk include “mild” \textit{APC} and mismatch repair gene mutations, as well as polymorphisms of genes involved in nutrient or carcinogen metabolism.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{incidences_of_colorectal_cancers}
\caption{Incidences of colorectal cancers.}
\end{figure}

Figure no. 10 depicts the blood supply to the colon. It can be seen that blood is derived from branches of the superior mesenteric artery (from the cecum to the splenic flexure) and the inferior mesenteric artery (descending colon, sigmoid and rectum). The lower rectum is supplied by the middle and inferior rectal arteries and
branches of the internal iliac artery. This vascularization lends to support to the suggestion that there are three different types of colorectal malignancies:

- Cancer of the right colon (i.e., neoplasms located from the cecum to the splenic flexure).
- Cancer of the left colon (tumours of the descending, sigmoid and rectosigmoid region).
- Cancer of the rectum (tumours located within 8 cm of the anal verge).

The large majority of the venous blood leaves the colon through the portal system and thus, reaches the liver— which is therefore, the main site of haematogenous metastasis from colorectal cancers.

Figure no. 10: Blood supply to the colon.
1.4 PATHOLOGY IN CRC

Figure no. 11 summarizes the distribution of the incidences of the cancer of the colon and rectum\textsuperscript{85}. Colorectal adenocarcinomas account for about 90\% of the malignant cancers of the large bowel. Two-thirds of these cancers are located in the rectum (38\%), rectosigmoid or sigmoid colon (20\%) with the other one-third distributed in the remainder of the colon. Many of these cancers begin as adenomatous polyps. The progression from adenoma to carcinoma occurs by sequential accumulation of genetic changes. Dysplasia, adenoma size (2cm), extent of villous component, multiplicity and increasing age are factors associated with a high potential for malignancy\textsuperscript{86}.

Figure no. 11: Typical sites of incidences of colon cancer.

1.5 STAGING IN CRC

It is recommended that the TNM staging system be applied uniformly to patients with colorectal cancer\textsuperscript{87-89}. The TNM classification of malignant tumours is a cancer staging system that describes the extent of the cancer in a patient’s body.

- T describes the size of the tumour and whether it has invaded nearby tissue,
- N describes the regional lymph nodes that are involved,
- M describes the distant metastasis.

Unlike other solid tumour TNM classifications, the T in colon and rectal cancer does not relate to the size of the lesion but rather to the depth of penetration by the tumour into or through the bowel wall. The actual TNM classification and its relationship to the most popular modification of the Dukes system (i.e., the Astler/Coller modification of Dukes/Kirklin) is summarized in table no. 1.
Table no. 1: Carcinoma of the Colorectum: Stage classification and stage grouping (AJCC, UICC, Dukes, Astler-Coller).

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<tbody>
<tr>
<td>Stage 0</td>
<td>Carcinoma in situ</td>
<td>Stage 0</td>
<td>Tis, NO, MO</td>
<td>Stage 0</td>
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<td></td>
<td>Tis, NO, MO</td>
<td>Tis, NO, MO</td>
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<tr>
<td>Stage I</td>
<td>1A Tumor confined to mucosa or submucosa</td>
<td>Stage I</td>
<td>1A</td>
<td>Stage 1</td>
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<td>T1, NO, MO</td>
<td>T1, NO, MO</td>
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<td></td>
<td>1B Tumor involves muscularis propria but not beyond T2, NO, MO</td>
<td>1B</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>T2, NO, MO</td>
<td>T2, NO, MO</td>
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<tr>
<td>Stage II</td>
<td>Involvement of all layers of bowel wall with or without invasion of immediately adjacent structures</td>
<td>Stage II</td>
<td>T3, T4, NO, MO</td>
<td>Stage II</td>
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<tr>
<td></td>
<td>T3, NO, MO</td>
<td>(T3a with fistula)</td>
<td></td>
<td>B2</td>
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<tr>
<td></td>
<td></td>
<td>(T3a without fistula)</td>
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<tr>
<td>Stage III</td>
<td>Any degree of bowel wall involvement with regional node metastasis.</td>
<td>Stage III</td>
<td>Any T, N1, MO</td>
<td>Stage III</td>
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<td></td>
<td>Any T, N1-N3; MO</td>
<td></td>
<td></td>
<td>C1</td>
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<td></td>
<td>Extends beyond contiguous tissue or immediately adjacent organs with no regional lymph node metastasis</td>
<td>C (1932)</td>
<td>C1 (1935)</td>
<td></td>
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<tr>
<td></td>
<td>T4, NO, MO</td>
<td>C2 (1935)</td>
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<td>C2</td>
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<tr>
<td>Stage IV</td>
<td>Any invasion of bowel wall with or without regional lymph node metastasis but with evidence of distant metastasis</td>
<td>Stage IV</td>
<td>Any T, any N, M1</td>
<td>Stage IV</td>
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<tr>
<td></td>
<td>Any T, any N, M1</td>
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<td>D</td>
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*Dukes A = Limited to bowel wall; B = spread to extramural tissue; C = involvement of regional nodes (C1: near primary lesion; C2: proximal node involved at point of ligation); type 4 (so-called D) = distant metastasis.

†Astler-Coller: A = Limited to mucosa; B1 = same as AJCC stage 1B (T2a); B2 = same as AJCC Stage 1B (T2b); C1 = limited to wall with involved nodes; C2 = through all layers of wall with involved nodes.
1.6 SIGNS AND SYMPTOMS OF CRC
Early stage colorectal cancer does not usually have symptoms; therefore, screening is of utmost importance to detect colorectal cancer in its early stages. Advanced disease may cause rectal bleeding, blood in the stools, a change in bowel habits and cramping pain in the lower abdomen. In some cases, blood loss from the cancer leads to anemia, causing symptoms such as weakness and excessive fatigue.

1.7 RISK FACTORS FOR CRC
The risk of colorectal cancer increases with age; 91% of cases are diagnosed in individuals aged 50 and older. Several modifiable factors are associated with increased risk of colorectal cancer. Among these are obesity, physical inactivity, a diet high in red or processed meat, heavy alcohol consumption and possibly smoking and inadequate intake of fruits and vegetables. Consumption of milk and calcium appears to decrease risk. Studies suggest that regular use of non-steroidal anti-inflammatory drugs, such as aspirin and menopausal hormone therapy may also reduce colorectal cancer risk. However, these drugs are not currently recommended for the prevention of colorectal cancer because they can have other serious adverse health effects. Colorectal cancer risk is also increased by certain inherited genetic mutations [familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC) or Lynch syndrome], a personal or family history of colorectal cancer and/or polyps, or a personal history of chronic inflammatory bowel disease. Studies have also found an association between diabetes and colorectal cancer.

1.8 SCREENING FOR CRC
Screening procedures for colon cancer can be divided into two categories:

a) For patients who fall in the high risk group, and
b) For those with no known risk factors other than age.

1.8.1 SCREENING HIGH-RISK INDIVIDUALS
Patients with a prior episode of colon or rectal cancer carry a significantly increased risk of developing a metachronous lesion. Often, these lesions are not cancerous, but are premalignant adenomatous polyps. Removal of these polyps can be done
endoscopically and can prevent cancer\textsuperscript{103}. Relatives of patients with colon cancer may have the familial polyp syndromes or the HNPCC\textsuperscript{104}. Such people may benefit from a screening study of the entire large bowel mucosa\textsuperscript{105}. For patients with familial polyposis coli (FPC), colonoscopy should start after adolescence when the phenotype is first expressed\textsuperscript{106}. Surgical intervention that can remove the large bowel mucosa at risk for malignant transformation should be undertaken. If ignored, these patients eventually develop colon or rectal cancer. The latency between diagnosis of polyposis and development of cancer is variable, averaging from 10-15 years. Once cancer has developed, it behaves stage for stage like sporadic colorectal carcinoma. Patients with Crohn’s disease also fall in the high-risk category. Here, the segment of the colon involved in the inflammatory process cannot be predicted. Removal of the inflamed bowel does not prevent the recurrence of Crohn’s disease nor does it reduce the potential for subsequent malignant transformation in other parts of the bowel. Hence, the option of surgery is unavailable for such patients. Patients with ulcerative colitis have a defined increased probability of intestinal epithelial transformation. Histological identification of conversion from mild to severe dysplasia indicates a need to remove the large bowel before the cancer develops\textsuperscript{107}.

1.8.2 SCREENING OF ASYMPTOMATIC POPULATIONS AT STANDARD RISK

Screening involves testing for presence of occult blood in the stools\textsuperscript{108, 109}. If positive, this should be immediately followed by sigmoidoscopy. Colonoscopy is preferred to fecal testing. It should be repeated every five years after the age of 50 and more often if polyps are found. Current screening recommendations include guaiac testing\textsuperscript{110}, sigmoidoscopy\textsuperscript{111} and colonoscopy\textsuperscript{112}. The stool guaiac test or guaiac fecal occult blood test (gFOBT) is one of several methods that detect the presence of fecal occult blood. “Guaiac” denotes a paper surface on which is embedded a phenolic compound \(\alpha\)-guaiaconic acid. The feces are applied to a thick paper onto which is attached a thin film coated with guaiac. Following this, one or two drops of hydrogen peroxide are dripped onto the other side of the film. The hydrogen peroxide oxidizes the \(\alpha\)-guaiaconic acid to a blue colored quinone. Normally, when no blood and no peroxidases or catalases (from food) are present, this oxidation occurs very slowly. Heme from hemoglobin (of occult blood) catalyzes this reaction, giving a result in
about two seconds. Therefore, a positive test result is one where there is a quick and intense blue color change of the film. An annual guaiac testing starting at 45 years of age and endoscopy starting at 50 years of age at 3- to 5-year intervals is recommended.

Figure no. 12: The endoscopic techniques for detecting polyps: a: Colonoscopy; b: Sigmoidoscopy.

Colonoscopy is an endoscopic examination of the colon. It allows examination of the entire colon, about 4-5 feet in length. Also, there is a scope of removal of polyps for biopsy. As against this, sigmoidoscopy allows an examination of the distal portion (final two feet) of the colon. A sigmoidoscopy is used as a screening procedure for a full colonoscopy, often done in conjunction with a fecal occult blood test (FOBT). About 5% of these screened patients are referred to colonoscopy.

1.8.3 TUMOUR MARKERS\textsuperscript{113,114}:

1.8.3.1 CARCINOEMBRYONIC ANTIGEN (CEA)

CEA is an acid glycoprotein (molecular weight 200,000) in the periphery of tumour cell membrane\textsuperscript{115}. It is most heavily concentrated on the luminal surface and is capable of being easily released into the surrounding body fluids\textsuperscript{116}. Its levels are elevated in plasma of patients in presence of tumours of the colon as well as breast,
lung, pancreas, ovary and other adenocarcinomas\textsuperscript{117-119}. Hence, measurement of CEA levels is an indirect evidence of tumour presence. CEA elevation correlates with the stage of the disease. Levels of CEA can also be used to follow the response of metastatic tumour to treatment. Rising levels indicate tumour progression, falling levels indicate regression.

\textbf{1.8.3.2 CA19.9 ANTIBODY AND MONOCLONAL ANTIBODY}
Carbohydrate antigen 19.9 is defined by the mouse monoclonal antibody 1116 NS 19.9, raised against a human colonic carcinoma cell line\textsuperscript{120}. When a tumour does not produce CEA, the 19.9 assay might serve as a substitute to the use of CEA for detecting recurrence and for serial monitoring. High serum concentrations of CA19.9 have been reported in patients with colorectal cancers.

\textbf{1.8.3.3 CATHEPSIN B}
Cathepsin B is a lysosomal protease that can degrade matrix components, thus attenuating tissue basement membranes. This results in a higher metastatic potential and a worse prognosis\textsuperscript{121, 122}. Increased expression of cathepsin B correlate with the stage of cancer and inversely with survival. Thus it is important to the progression from benign/ low-grade neoplasm to an invasive/ metastatic malignancy.

\textbf{1.9 EPIDEMIOLOGY}
In a year, a little over half a million new cases of colorectal cancer have been estimated to occur globally. Even with the assumption of no change in the incidence/ mortality rates over the decades, the absolute numbers have increased because of a steady increase in world population and its progressive ageing, with major implications for cancer control. Globally, colorectal cancer is the third commonest cancer in men since 1975\textsuperscript{123-127}. In the developed countries, it is now the second most common cancer after lung cancer in men and the 1990 age-standardized incidence, rates range from 25.3 per 100,000 (Eastern Europe)\textsuperscript{128} to 45.8 per 100,000 (Australia). Incidence rates in Africa, except South Africa and South and Central Asia including India are quite low (2 to 8 per 100,000), though they have been on the increase in the recent past\textsuperscript{129-131}.
Colorectal cancer burden has been steadily rising in women (table no. 2). It was the fourth commonest cancer in 1975 and had reached the second position by 1990, with about 49% increase in the number of cases globally over the 15 years. As in men (table no. 3), the disease is more common in women of the developed countries than in the developing countries of Asia and Africa. In fact almost two-thirds of the estimated world total of 783,000 new cases in 1990 occurred in the developed countries (figure nos. 13 and 14).

Table no. 2: Estimated number of new cancer cases (thousands) worldwide in women, ranked (in parentheses) by 1990 order.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast (174)</td>
<td>541.2</td>
<td>572.1</td>
<td>719.1</td>
<td>795.6</td>
</tr>
<tr>
<td>Colon and rectum (153-154)</td>
<td>255.6</td>
<td>285.9</td>
<td>346.5</td>
<td>381.0</td>
</tr>
<tr>
<td>Cervix (180)</td>
<td>459.4</td>
<td>465.6</td>
<td>437.3</td>
<td>371.2</td>
</tr>
<tr>
<td>Stomach (151)</td>
<td>260.6</td>
<td>260.6</td>
<td>282.3</td>
<td>287.2</td>
</tr>
<tr>
<td>Lung (162)</td>
<td>126.7</td>
<td>146.9</td>
<td>219.3</td>
<td>265.1</td>
</tr>
<tr>
<td>Ovary (183)</td>
<td>NA (&gt;11)</td>
<td>137.6</td>
<td>161.5</td>
<td>165.5</td>
</tr>
<tr>
<td>Corpus uteri (182)</td>
<td>NA (&gt;11)</td>
<td>148.8</td>
<td>140.0</td>
<td>142.4</td>
</tr>
<tr>
<td>Liver (155)</td>
<td>76.7</td>
<td>79.4</td>
<td>100.7</td>
<td>121.1</td>
</tr>
<tr>
<td>Mouth and pharynx(140-149)</td>
<td>106.6</td>
<td>121.2</td>
<td>142.8</td>
<td>105.4</td>
</tr>
<tr>
<td>Oesophagus (150)</td>
<td>102.3</td>
<td>108.2</td>
<td>107.6</td>
<td>103.2</td>
</tr>
<tr>
<td>All sites (140-208)</td>
<td>2901.8</td>
<td>3103.1</td>
<td>3774.2</td>
<td>3789.8</td>
</tr>
</tbody>
</table>

Figure no. 13: Estimated number of new cases of cancer (in thousands) in developed and developing countries (females), 1985 and 1990.
Introduction

From a total of 1,596,670 new cancer cases and 571,950 deaths from cancer recorded in the U.S. in 2011, overall death rates decreased by about 22% in men and 14% in women\textsuperscript{132}. Cancers of the lung, prostate, and colon/rectum in men, and cancers of the lung, breast, and colon/rectum in women continue to be the most common causes of cancer death. These four cancers account for almost half of the total cancer deaths among men and women\textsuperscript{133}. The incidence rates of both large and small bowel cancer are low in India, and rectal cancer is more common than colon cancer\textsuperscript{134}. The incidence rates of colon cancer in eight population registries vary from 3.7 to 0.7/100,000 among men and 3 to 0.4/100,000 among women. For rectal cancer the incidence rates range from 5.5 to 1.6/100,000 among men and 2.8 to 0/100,000 among women. One intriguing observation is the occurrence of rectal cancer in young Indians. Rural incidence rates for large bowel cancers in India are approximately half of urban rates. Significant increase in the incidence of colon cancer has been reported for both men and women over two decades, but the rates of rectal cancer are steady. The role of hereditary factors has been evaluated in a few studies. Some studies have reported the occurrence of both FAP and HNPCC in India.

Table no. 3: Estimated number of new cancer cases (thousands) worldwide in men, ranked (in parentheses) by 1990 order.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung (162)</td>
<td>464.3(1)</td>
<td>513.6(1)</td>
<td>676.5(1)</td>
<td>771.8(1)</td>
</tr>
<tr>
<td>Stomach (151)</td>
<td>421.7(2)</td>
<td>408.8(2)</td>
<td>472.5(2)</td>
<td>510.0(2)</td>
</tr>
<tr>
<td>Colon and rectum (153-154)</td>
<td>251.2(3)</td>
<td>286.2(3)</td>
<td>331.0(3)</td>
<td>401.9(3)</td>
</tr>
<tr>
<td>Prostate (185)</td>
<td>197.7(5)</td>
<td>235.8(5)</td>
<td>291.2(4)</td>
<td>396.1(4)</td>
</tr>
<tr>
<td>Liver (155)</td>
<td>182.5(7)</td>
<td>171.7(7)</td>
<td>214.2(6)</td>
<td>316.3(5)</td>
</tr>
<tr>
<td>Mouth and pharynx (140-149)</td>
<td>232.9(4)</td>
<td>257.3(4)</td>
<td>269.6(5)</td>
<td>257.7(6)</td>
</tr>
<tr>
<td>Oesophagus (150)</td>
<td>194.0(6)</td>
<td>202.1(6)</td>
<td>195.9(7)</td>
<td>212.6(7)</td>
</tr>
<tr>
<td>Bladder (188)</td>
<td>130.7(8)</td>
<td>167.7(8)</td>
<td>181.7(8)</td>
<td>202.5(8)</td>
</tr>
<tr>
<td>Lymphoma (200-203)</td>
<td>129.5(9)</td>
<td>139.9(9)</td>
<td>180.8(9)</td>
<td>192.5(9)</td>
</tr>
<tr>
<td>Leukemia (204-208)</td>
<td>100.3(10)</td>
<td>106.9(10)</td>
<td>120.5(10)</td>
<td>130.3(10)</td>
</tr>
<tr>
<td>All sites (excluding skin) (140-208)</td>
<td>2968.5</td>
<td>3246.6</td>
<td>3849.4</td>
<td>4293.5</td>
</tr>
</tbody>
</table>
Figure no. 14: Estimated number of new cases of cancer (in thousands) in developed and developing countries (males), 1985 and 1990.

1.10 TREATMENT MODALITIES
The four main types of treatment for colorectal cancer are:  
- Surgery  
- Radiation therapy  
- Chemotherapy  
- Targeted therapies using monoclonal antibodies

Surgery is the most common treatment for colorectal cancer. For cancers that have not spread, surgical removal may be curative. A permanent colostomy (creation of an abdominal opening for elimination of body wastes) is rarely needed for colon cancer and is infrequently required for rectal cancer. Chemotherapy alone, or in combination with radiation (for rectal cancer), is given before or after surgery to most patients whose cancer has penetrated the bowel wall deeply or spread to lymph nodes. Adjuvant chemotherapy (anticancer drugs in addition to surgery or radiation) for colon cancer is equally effective and can be no more toxic in otherwise healthy patients aged 70 and older than in younger patients. Oxaliplatin, in combination with 5-fluorouracil (5-FU) and followed by leucovorin (LV), may be used to treat persons with metastatic carcinoma of the colon or rectum. Three targeted monoclonal antibody therapies are used to treat metastatic colorectal cancer: bevacizumab blocks
the growth of blood vessels to the tumour and cetuximab and panitumumab both block the effects of hormone-like factors that promote cancer cell growth.

1.10.1 CHEMOPREVENTION OF CRC
As seen in section 1.3, a genetic model of colon carcinogenesis has been observed and it is well known that this cancer results not from any single genetic event but from the accumulation of a number of genetic alterations. By interfering with these molecular events, chemoprevention could inhibit or reverse the development of adenomas or the progression from adenoma to cancer. This approach has proved useful not only in patients with FAP but also in persons with no known genetic syndrome but with a history of sporadic polyps.

1.10.1.1 ASPIRIN AND OTHER NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs)
It is likely that aspirin and other NSAIDs act as chemopreventive agents at the early stages of carcinogenesis. These are the most widely studied agents for the chemoprevention of CRC. They exert their effect by a number of mechanisms:

- They inhibit both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), the catalytic enzymes involved in prostaglandin synthesis. COX-1 is constitutively expressed whereas COX-2 is induced by cytokines, mitogens and growth factors. It is seen that COX-2 levels are elevated in 90% of sporadic colon carcinomas and 40% colon adenomas but is not elevated in normal colonic epithelium. Hence, when treated with NSAIDs such as sulindac (inhibitor of both COX-1 and COX-2), the number of intestinal adenomas decreases by 90%. Inhibition of COX-2 leads to an increase in arachidonic acid, which in turn, stimulates the conversion of sphingomyelin to ceramide, a mediator of apoptosis, which may eventually lead to decreased colon carcinogenesis by causing an increase in apoptosis, regulation of angiogenesis or both. It may also lead to apoptosis by altering prostaglandin production and by decreasing angiogenic factors.

- Aspirin and NSAIDs may also act by COX-independent mechanisms, such as inhibition of activation of nuclear factor-κB (NF-κB).
• These may also interfere with the binding of the peroxisome-proliferator-activated receptor δ (PPARδ) to DNA. However, it is important to note that long-term ingestion of aspirin and NSAIDs causes gastric irritation and platelet dysfunction, which may be due to inhibition of COX-1.

![Colon carcinogenesis and the effects of chemotherapeutic agents](image)

**Figure no. 15: Colon carcinogenesis and the effects of chemotherapeutic agents.**

The various chemopreventive agents exert their effects at different stages in this pathway, and this is depicted on the basis of the available epidemiologic evidence. NSAIDs denotes non steroidal anti-inflammatory drugs, COX-2 cyclooxygenase-2 and APC the adenomatous polyposis coli gene.

**1.10.1.2 FOLATE AND CALCIUM**

Epidemiologic studies have found a lower incidence of CRC among those with high dietary folate intake. The mechanisms through which folic acid acts to inhibit tumourigenesis are unknown. Also, diets rich in red meat and animal fat are associated with an increased risk of colorectal adenomas and cancer. Although the exact mechanism is not known, these diets increase the production of secondary bile acids, which may cause hyperproliferation of the colorectal epithelium and which promote tumour formation. Calcium may inhibit colon tumourigenesis by binding bile
acids and fatty acids in the colon lumen or by directly inhibiting the proliferation of the colonic epithelial cells\textsuperscript{144}.

\textbf{Figure no. 16: Mechanisms of action of aspirin, other NSAIDs, and selective COX-2 inhibitors.} Plus sign indicates stimulation or activation and minus sign, inhibition.

\textbf{1.10.1.3 HORMONE REPLACEMENT THERAPY}
In the past two decades, mortality from CRC has decreased slightly in men but much more in women. A possible explanation for this may be the increased use of postmenopausal hormone-replacement therapy\textsuperscript{145}. Estrogens may prevent CRC by decreasing the production of secondary bile acids, decreasing the production of insulin-like growth factor I, exerting direct effects on the colorectal epithelium or by a combination of these mechanisms.

\textbf{1.10.2 CHEMOTHERAPY OF CRC}
Despite curative surgical resection in 70-80\% of patients, nearly 40\% of them either present with metastases or develop disease recurrence and eventually die. In these patients, chemotherapy seems to be the only therapeutic modality which may offer some benefit\textsuperscript{146}. 5-Fluorouracil (5-FU) plus levamisole reduce the recurrence levels
by 40%. For patients with advanced disease, 5-FU in combination with folinic acid (leucovorin) improves response as compared to 5-FU alone\textsuperscript{147-149}. With the introduction of chemotherapeutic agents such as capecitabine, oxaliplatin and irinotecan, the mean overall survival of patients with advanced stage disease has improved\textsuperscript{150}. Anti-angiogenic drugs such as bevacizumab (monoclonal antibody targeting vascular endothelial growth factor VEGF) and cetuximab (monoclonal antibody targeting epidermal growth factor receptor EGFR) when combined with chemotherapy have shown to improve survival in patients with advanced disease\textsuperscript{151}. The ability of chemotherapeutic agents to induce apoptosis appears to be an important determinant of sensitivity/resistance to cytotoxic therapies.

1.11 DRAWBACKS OF CURRENT TREATMENT

Though the mean survival rates of patients suffering from colorectal carcinomas are better than those suffering from other cancers, the treatment modalities used are not without side effects. Each option needed to alleviate the disease comes with its share of adverse effects\textsuperscript{152}.

- Side effects of surgery depend on several considerations, such as the extent of the operation and a person's general health before surgery. Possible side effects of surgery include bleeding, blood clots in the legs, and damage to nearby organs during the operation. Rarely, the connections between the ends of the intestine may not hold together completely and leak, which can lead to infections. Some people may need a short-term or permanent colostomy (or ileostomy) after surgery.

- Side effects of radiation therapy for colon or rectal cancer include skin irritation, nausea, diarrhoea, trouble controlling bowel movement, rectal or bladder irritation and tiredness.

- The side effects of chemotherapy depend upon the type, combination and schedule of drug used. The side effects of the drug combinations used are as follows:

  - 5-FU and leucovorin: Common side effects are diarrhea, mucositis, bone marrow depression and photosensitivity. It is known that women experience more severe toxicity than men while receiving bolus 5-FU based chemotherapy\textsuperscript{153}.
• **Irinotecan:** When given alone, irinotecan usually causes more diarrhea, lower blood counts, fatigue and hair loss.

• **Oxaliplatin:** It can cause numbness and tingling of the hands and feet. It also causes an unusual sensitivity to cold temperatures. It can result in painful spasms of the throat.

• **Bevacizumab:** It may rarely cause allergic responses. It can also cause an impairment of wound healing. Hence surgeries should be avoided if this drug is being used. It may cause bleeding in the GI tract and there is an increased risk of blood clotting. It can also increase blood pressure and cause proteinurea. About 5% patients have serious side effects such as strokes and heart attacks during therapy.

• **Capecitabine:** The most common side effect of capecitabine is hand-foot syndrome. In this, there is soreness, redness and peeling of skin of the palms and soles of the feet.

• **Cetuximab:** It causes allergic reactions. Other side effects include skin rash and low blood levels of magnesium (which in turn causes heart rhythm abnormalities).

1.12 **SURVIVAL**

The 1- and 5-year relative survival for persons with CRC is 83% and 64%, respectively. Survival continues to decline beyond 5 years to 58% at 10 years after diagnosis. When colorectal cancers are detected at an early, localized stage, the 5-year survival is 90%; however, only 40% of colorectal cancers are diagnosed at this stage, mostly due to underuse of screening.\(^{154}\)

1.13 **PLANTS AS A SOURCE OF NOVEL MOIETIES**

The development of resistance by tumour cells to chemotherapeutic agents is a major problem in cancer therapy. One way to counter this is to find compounds with cytotoxic mechanisms other than those of drugs in clinical use today. The biological and chemical diversity encountered in nature provides opportunities to discover completely new classes of compounds.\(^{155}\) Some of these may represent previously
unknown anticancer agents and in some cases, novel, potentially relevant cytotoxic mechanisms\textsuperscript{155-165}. The great varieties of secondary metabolites from plants have been sources of commercially important pharmaceutical compounds. A systematic effort is now being undertaken globally to screen natural products; herbal medicines in particular, for various pharmacological activities, especially anticancer with an aim to isolate novel compounds with unique mechanism/s of action\textsuperscript{166-180}.

Anticancer drugs of natural origin and their semisynthetic analogues exert their effects on the cancer cell with distinct definable mechanisms. For example, the topoisomerase inhibitors (e.g. podophyllotoxin, topotecan, etoposide) interfere with transcription, DNA synthesis and mitosis by blocking the enzyme DNA topoisomerase I and II. In contrast, the vinca alkaloids and taxanes e.g. vincristine and paclitaxel, block the polymerization and depolymerisation, respectively of microtubule, thereby interfering with key steps in the cell division, such as organization of the mitotic spindle and thus, the mitotic arrangement of the chromosomes.

Camptothecin promotes DNA strand breaks, thus disturbing DNA replication, whereas anthracyclines, including doxorubicin and daunorubicin, have multiple sites of action that include intercalation into the DNA, inhibition of DNA topoisomerase II, production of free radicals and binding to the plasma membranes.
### Table no. 4: Examples of anticancer substances of natural origin, their origin, therapeutic use and a brief description of their mechanism of action.

<table>
<thead>
<tr>
<th>Name of the compound</th>
<th>Origin</th>
<th>Therapeutic use</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camptothecin&lt;sup&gt;187&lt;/sup&gt;</td>
<td><em>Camptotheca acuminate</em></td>
<td>Gastrointestinal tumours</td>
<td>Enhances the binding of topoisomerase I to DNA, promotes DNA strand breaks.</td>
</tr>
<tr>
<td>Docetaxel&lt;sup&gt;188-190&lt;/sup&gt;</td>
<td><em>Taxus baccata</em></td>
<td>Ovarian, breast and bronchial carcinomas</td>
<td>Binding to tubulin sub-units and stabilization of microtubule.</td>
</tr>
<tr>
<td>Etoposide, Teniposide&lt;sup&gt;191, 192&lt;/sup&gt;</td>
<td>Semisynthetic podophyllotoxin derivative from <em>Podophyllum</em> sp.</td>
<td>SCLC, leukaemia, non-Hodgkin lymphoma, Hodgkin’s disease and testicular cancer</td>
<td>DNA Topoisomerase II inhibitor.</td>
</tr>
<tr>
<td>Irinotecan, topotecan&lt;sup&gt;193&lt;/sup&gt;</td>
<td>Semisynthetic, synthesized from camptothecin</td>
<td>Colorectal, ovarian carcinomas respectively</td>
<td>DNA Topoisomerase I inhibitor.</td>
</tr>
<tr>
<td>Paclitaxel&lt;sup&gt;194&lt;/sup&gt;</td>
<td><em>Taxus brevifolia, taxus cuspidate</em></td>
<td>Breast carcinomas, metastasizing NSCLC.</td>
<td>Binding to tubulin sub-units, stabilization of microtubule.</td>
</tr>
</tbody>
</table>

There is an increased use of herbs along with conventional drugs rather than using them in place of drugs<sup>197</sup>. This may be due to the following reasons:

i. Increase in the cost of health care, drug prices and number of patients.

ii. Increase in multidrug resistance which has led to a search for alternative modes of treatment.

iii. The decreased efficacy and treatment failure of modern drugs favours the use of herbal medicines.

iv. Due to the complex interconnected nodes of cell signaling network, it is important to use multiple modulating strategies to achieve clinical success.
Phytomedicines can achieve this by exerting beneficial effects through additive or synergistic actions of several chemical compounds acting at single or multiple target sites associated with a physiological process. A number of signal transduction pathways in cancer are known to be blocked by phytochemicals. Some of these are as shown in table no. 5.

Table no. 5: Examples of some phytoconstituents used in anticancer therapy and their mechanism of action.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Phytochemicals</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Curcumin&lt;sup&gt;198&lt;/sup&gt;, Capsaicin&lt;sup&gt;199&lt;/sup&gt;, Resveratrol&lt;sup&gt;200&lt;/sup&gt;, Emodin&lt;sup&gt;201&lt;/sup&gt;, Sanguinarine&lt;sup&gt;202&lt;/sup&gt;, Flavopyridol&lt;sup&gt;203&lt;/sup&gt;</td>
<td>Inhibition of activation of NF-κB pathway</td>
</tr>
<tr>
<td>2.</td>
<td>Curcumin, Capsaicin, Resveratrol, Green tea catechins&lt;sup&gt;204&lt;/sup&gt;</td>
<td>Inhibition of AP-1 activation pathway</td>
</tr>
<tr>
<td>3.</td>
<td>Curcumin&lt;sup&gt;205&lt;/sup&gt;, Resveratrol, EGCG&lt;sup&gt;206&lt;/sup&gt;, Genistein</td>
<td>Inhibition of Tyrosine kinase activity of EGRF</td>
</tr>
<tr>
<td>4.</td>
<td>Curcumin&lt;sup&gt;207&lt;/sup&gt;, EGCG&lt;sup&gt;208&lt;/sup&gt;</td>
<td>Inhibition of multidrug resistant related proteins</td>
</tr>
<tr>
<td>5.</td>
<td>Curcumin&lt;sup&gt;209&lt;/sup&gt;, Resveratrol&lt;sup&gt;210&lt;/sup&gt;, EGCG&lt;sup&gt;211&lt;/sup&gt;</td>
<td>Blocking of cell cycle at various phases</td>
</tr>
<tr>
<td>6.</td>
<td>Curcumin&lt;sup&gt;212&lt;/sup&gt;, Resveratrol&lt;sup&gt;213&lt;/sup&gt;, Genistein, Catechin</td>
<td>Inhibition of induction of COX-2</td>
</tr>
<tr>
<td>7.</td>
<td>Flavanoids&lt;sup&gt;214&lt;/sup&gt;, EGCG&lt;sup&gt;215&lt;/sup&gt;</td>
<td>Inhibition of Receptor tyrosine kinase (RTK) related pathways of signal transduction</td>
</tr>
<tr>
<td>8.</td>
<td>Curcumin&lt;sup&gt;214,216&lt;/sup&gt;</td>
<td>Blocking of phosphatidyl inositol-3- kinase pathway</td>
</tr>
</tbody>
</table>

1.14 REACTIVE OXYGEN SPECIES- A DOUBLE-EDGED SWORD

"Reactive oxygen species" (ROS) is a collective term that describes chemical species that are formed upon incomplete reduction of oxygen and have the ability to react with reducible compounds<sup>217</sup>. They comprise of superoxide radical (O<sub>2</sub><sup>-</sup>·), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and the highly reactive hydroxyl radical (·OH), although minor amounts of singlet oxygen can also be formed by cells<sup>218</sup>. About 5% of the inhaled oxygen is converted to free radicals. Thus, cells under aerobic conditions are always threatened with the insult of ROS. Initial product of the electron transport chain is
O$_2^-$, which is quickly transformed into H$_2$O$_2$ by the enzyme superoxide dismutase (SOD)\textsuperscript{219}. The latter reduces the O$_2^-$ to water by catalase or glutathione peroxidase or can be converted into ·OH in presence of reduced transition metals (reduced copper or iron). ROS are also continuously produced as byproducts of different metabolic pathways localized in various cellular compartments. They are thought to mediate the toxicity of oxygen because of their greater chemical reactivity with respect to oxygen. Under physiologic steady state conditions, these molecules are efficiently scavenged by different antioxidative defence components that are confined to particular compartments. The equilibrium between production and scavenging of ROS may be perturbed by a number of factors. As a result of these disturbances, intracellular levels of ROS may rise rapidly. This rapid increase in ROS concentration is called “oxidative burst”. Cells also generate ROS by activating various oxidases and peroxidases in response to certain environmental changes.

A common feature among the different ROS types is their capacity to cause oxidative damage to proteins, DNA and lipids\textsuperscript{220}. These, through a series of events deregulate the cellular functions leading to various pathological conditions such as cardiac dysfunction, neurodegenerative diseases, cancer, ageing etc. ROS-mediated DNA damage has long been thought to play a role in carcinogenesis initiation and malignant transformation\textsuperscript{221}. Hydroxyl radicals, for example, react with pyrimidines, purines and chromatin protein, resulting in base modifications, genomic instability and alterations in gene expression. Mitochondrial DNA is a particularly vulnerable target because of its proximity to the electron transport chain constituents. ROS-mediated mutations in mitochondrial DNA have recently emerged as an important variable in carcinogenesis\textsuperscript{222}.

The cytotoxic properties of ROS explain the evolution of complex array of enzymatic and non-enzymatic detoxification mechanisms. On one hand, the cells are equipped with mechanisms to combat increased ROS levels, in other circumstances, cells purposefully generate ROS as intracellular signaling molecules to control various processes including pathogen defence and programmed cell death\textsuperscript{223}.
Introduction

Figure no. 17: Chronic ROS exposure is carcinogenic. Excess levels are toxic to cancer cells.

1.15 MITOCHONDRION-THE POWER PACKED SUICIDE MACHINE
Mitochondria are cellular organelles intimately involved in energy production and are essential for cellular life\textsuperscript{224, 225}. These organelles also play an important role in cell death which occurs when their membranes are permeabilized. Mitochondria possess two discreet membrane systems, an outer membrane in close communication with the cytosol and an inner membrane involved in ion transport, protein import, biogenesis and energy production. Although both membranes can be involved in the permeabilization process, the molecular mechanisms regulating inner membrane permeabilizations are fundamentally different from those regulating outer membrane permeabilization. Since the mode of death; apoptosis or necrosis depends on which membrane is permeabilized, it is important to understand the molecular basis of such membrane permeabilization. This will help to design better therapeutic agents for use in diseases such as cancer.
1.15.1 APOPTOSIS AND CASPASE-INDEPENDENT CELL DEATH DUE TO MITOCHONDRIAL OUTER MEMBRANE PERMEABILIZATION

Apoptotic stimulation occurs through distinct signaling cascades\textsuperscript{226-228}. The intrinsic (or mitochondrial) pathway integrates signals generated by a variety of stressors, including DNA damage, cytoskeletal damage, endoplasmic reticulum stress, loss of adhesion, growth factor withdrawal, macro molecular synthesis inhibition and others. The characteristics of apoptosis (e.g., DNA laddering and chromatin condensation, loss of plasma membrane asymmetry and blebbing) are dependent on the activation of cysteine proteases (the caspases) that cleave numerous specific cellular substrates. The activation of caspases in the mitochondrial pathway requires mitochondrial outer membrane permeabilization (MOMP), an event that is considered to be the ‘point of no return’ during apoptosis as it results in the diffusion to the cytosol of numerous proteins that normally resides in the space between the outer (OMM) and inner (IMM) mitochondrial membranes. Among these is cytochrome c, which serves as a co-factor for apoptotic protease-activating factor-1 (APAF-1) to trigger the formation of the apoptosome and the subsequent activation of the initiator and executioner caspases, normally caspase-9 and -3 respectively.
In order for a mitochondrion to undergo MOMP, a co-ordinated effort between numerous Bcl-2 proteins must be engaged to allow for permeabilization of the OMM. This permeabilization is likely achieved by the formation of membrane-spanning pores through which the intermembrane space proteins are released. Once MOMP has occurred, the cytosolic machinery responds by activating caspases, or if this pathway is inhibited, a caspase-independent cell death (CICD) process ensures cell demise. This process may utilize less tractable mechanisms, such as ROS, loss of mitochondrial function or release of mitochondrial intermembrane space proteins such as apoptosis-inducing factor or endonuclease G to catalyze death\textsuperscript{229}. In both situations, caspase-dependent or -independent death, MOMP occurs. This disrupts the mitochondrial function, and even in case of caspase activation, energy production eventually wanes and the cell is left to die\textsuperscript{230}.

Figure no. 19: The mitochondrial pathway of apoptosis induction.
Between the outer and inner membrane space lies the intermembrane space, where proapoptotic proteins such as cytochrome c are located. The constituents of the electron transport chain, except for cytochrome c, are embedded in the inner membrane. Voltage-dependent anion channel (VDAC) is found exclusively in the outer membrane; this channel allows diffusion of metabolites and ions across the outer membrane. In contrast, the inner membrane is impermeable, allowing for the maintenance of a transmembrane potential, $\Delta \psi_m$. The inner membrane contains transport molecules, e.g., the adenine nucleotide transporter (ANT), responsible for the exchange of specific small molecules. Within the inner membrane is the matrix. Most mitochondrial proteins are encoded by nuclear genes and imported from the cytoplasm, through one or both membranes, via transport complexes Tom and Tim. The respiratory electron-transport chain and the ATP-synthase complex, both localized at the IMM are responsible for ATP synthesis and oxidative phosphorylation. The energy released by the transfer of electrons is used to pump out the $H^+$ from the mitochondria. Therefore, $H^+$ electrochemical potential (proton motive force; $\Delta p$) is formed by a proton gradient $\Delta p_H$ and an electrical gradient $\Delta \psi_m$. To produce ATP, the ATP-synthase complex mediates the $H^+$ flow back to the matrix and the energy is conserved in the phosphorylation of ADP to ATP. In fact, the $\Delta p$ is one of the most important physiological parameters that reflects energy status and
mitochondrial membrane transport\textsuperscript{231, 232}. Mitochondria are the only cell organelles known to have significant membrane potential, with a negative charge inside. The Nernst equation predicts a $\Delta \psi_m$ of -180mV. They utilize oxidizable substrates to produce a membrane potential in the form of a proton gradient across the mitochondrial inner membrane\textsuperscript{233}. It has been shown that the supply of oxidizable substrates to mitochondria depends on the concentration of external growth factors. If withdrawal of growth factors or glucose deprivation persists, cells ultimately undergo apoptosis that is initiated by cytochrome $c$ release from the mitochondria\textsuperscript{234}.

1.16 CELL CYCLE AND GROWTH INHIBITORS

![Cell growth cycle diagram](image)

*Figure no. 21: The cell growth cycle.*

The eukaryotic cell cycle is divided into four stages: G1, S, G2, and M. G1 is the gap phase during which cells prepare for the process of DNA replication\textsuperscript{235, 236}. It is during the G1 phase that the cell integrates mitogenic and growth inhibitory signals and makes the decision to proceed, pause, or exit the cell cycle. An important checkpoint in G1 has been identified in mammalian cells. Referred to as the restriction point, this is the point at which the cell becomes committed to DNA replication and completing
a cell cycle. S phase is defined as the stage in which DNA synthesis occurs. G2 is the second gap phase during which the cell prepares for the process of division. M stands for mitosis, the phase in which the replicated chromosomes are segregated into separate nuclei and cytokinesis occurs to form two daughter cells. In addition to G1, S, G2, and M, the term G0 is used to describe cells that have exited the cell cycle and become quiescent.

Cells can arrest at cell cycle checkpoints temporarily to allow for:

i. cellular damage to be repaired,
ii. the dissipation of an exogenous cellular stress signal or,
iii. availability of essential growth factors, hormones, or nutrients.

Checkpoint signaling may also result in activation of pathways leading to programmed cell death if cellular damage cannot be properly repaired. Defects in cell cycle checkpoints can result in gene mutations, chromosome damage and aneuploidy, all of which can contribute to tumourigenesis.

Several genes encoding regulatory activities that govern the cell cycle, particularly the progression of quiescent cells through G1 and into S phase, are targets for genetic and epigenetic alterations that underlie the development of many human neoplasias. The best characterized of these is cyclin D1. It is also now clear that cyclin D1 is the Bcl-1 oncogene, the gene involved in the t(11;14) (q13;q32) translocation associated with certain B-cell lymphomas.

1.16.1 MODULATION OF CELL CYCLE AS A THERAPEUTIC TARGET

The disruption of normal cell cycle regulation, which is the hallmark of cancer, presents numerous opportunities for targeting checkpoint controls to develop new therapeutic strategies for this disease. Such strategies include induction of checkpoint arrest leading to cytostasis and ultimately apoptosis, arrest of proliferating cells in stages of the cell cycle which may sensitize them to treatment with other therapeutic agents such as radiation, and targeting of therapies toward specific regulatory components of the cell cycle. Chemotherapeutic agents intervene at multiple points in the cell cycle. These drugs have diverse mechanisms of action and exhibit specificity in terms of the stage of the cell cycle in which they have activity. In table no. 6, several classes of chemotherapeutic agents and their mechanisms of action are listed along with information regarding their effects on the cell cycle. One of the most established chemotherapeutic approaches is the induction of DNA damage and
subsequent induction of apoptosis. Agents such as cisplatin and nitrogen mustard, which induce DNA cross-links and chromosome breakage, can cause cell cycle arrest at both the G1/S and G2/M checkpoints.\(^{237-239}\) The G2/M checkpoint induced by DNA damage can occur by either p53-dependent or independent mechanisms.\(^{240, 241}\) Microtubule inhibitors such as taxol and vinca alkaloids disrupt normal tubulin polymerization/depolymerization and mitotic spindle formation.\(^{242, 243}\) As a result, cells either initiate a p53-dependent arrest at the mitotic spindle assembly checkpoint, a radiosensitive phase of the cell cycle, or continue to progress through M and become aneuploid and arrest in G1.\(^{244, 245}\) Arrest in G2/M produced by these drugs is associated with stabilization of cyclin B/cdc2 complexes. Tumour cells treated with microtubule inhibitors can undergo apoptosis from both G1 and G2 arrest.\(^{246}\) Microtubule inhibitors have also proven effective in the clinic as radiosensitizers.

**Table no. 6: Intervention of therapeutic agents in the cell cycle.**

<table>
<thead>
<tr>
<th>Class of compounds</th>
<th>Mechanism of action</th>
<th>Prototypical drugs</th>
<th>Cell cycle impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA damaging agents</td>
<td>Induction of DNA alkylation and cross links; Clastogenic</td>
<td>Cisplatin, Nitrogen mustards, Cyclophosphamide, Chlorambucil</td>
<td>p53 mediated G1/S arrest/apoptosis G2/M arrest. Up-regulation of p21 and sequestration of PCNA</td>
</tr>
<tr>
<td>Microtubule inhibitors</td>
<td>Inhibition of tubulin polymerization, disruption of spindle formation</td>
<td>Taxol/ Paclitaxel, Nocodazole, Vincristine/ Vinblastine</td>
<td>Arrest at the mitotic spindle assembly checkpoint associated with stabilization of cyclin B/cdc2</td>
</tr>
<tr>
<td>Ribonucleotide pool depletion</td>
<td>Purine nucleoside analogs that inhibit DNA polymerase, Ribonucleotide reductase DNA chain elongation</td>
<td>Hydroxyurea, Gemcitabine, Difluorodeoxyuridine</td>
<td>p53 mediated up-regulation of p21 and arrest at G1 checkpoint. Cell killing in checkpoint defective cells that proceed into S.</td>
</tr>
<tr>
<td>Antimetabolites</td>
<td>Inhibition of thymidylate synthase and DNA synthesis</td>
<td>Methotrexate, Cytosine arabinoside, 5-Fluorouracil</td>
<td>p53 mediated S-phase arrest, apoptosis in checkpoint defective cells that incorporate antimetabolites.</td>
</tr>
<tr>
<td>Topoisomerase inhibitors</td>
<td>Inhibition of DNA topoisomerase and DNA synthesis</td>
<td>Camptothecin, Etoposide, Bufalin</td>
<td>S-phase damage resulting at arrest in S-phase or G2/M checkpoints. Up-regulation of p16 and arrest at G1/S checkpoint.</td>
</tr>
</tbody>
</table>
Several recent studies show that tumour cells with defective checkpoint function are more vulnerable to anticancer agents. In fact, the majority of human carcinomas have defective checkpoint function. Indeed, many laboratories are now searching for compounds that interfere with cell cycle checkpoints, in the hope that such agents will be more effective as anticancer therapy.

Also, an understanding of the cell cycle targets of different chemotherapeutic agents has prognostic implications and can have significant consequences for the development of resistance of tumours to chemotherapy and tumour evolution. As the understanding of cell cycle regulation and checkpoints increases so will the number of signaling molecules and pathways that are altered by xenobiotics and that can be used as targets for rational drug design.

1.17 COLORECTAL ADENOCARCINOMA CELL LINES USED

**HT-29**

*Figure no. 22: HT-29, high density.*

ATCC number: HTB-38
Organism: *Homo sapiens*
Source: Epithelial
Organ: Colon
Disease: Colorectal adenocarcinoma
Isolation: Isolation date: 1964
Age: 44 years adult
Gender: Female
Ethnicity: Caucasian

Ultra structural features reported for HT-29 include microvilli, microfilaments, large vacuolated mitochondria with dark granules, smooth and rough endoplasmic reticulum with free ribosomes, lipid droplets, few primary and many secondary lysosomes. The cells express urokinase receptors, but do not have detectable plasminogen activator activity. HT-29 cells are negative for CD4, but there is cell surface expression of galactose ceramide (a possible alternative receptor for HIV). The line is positive for the expression of k-ras, H-ras, N-ras, c-myc, Myb, sis and Fos oncogenes. The p53 antigen is overproduced, and there is a G to A mutation in codon 273 of the p53 gene resulting in an Arg to His substitution.

Colo205

![Image of Colo205, low density.](image)

**Figure no. 23: Colo205, low density.**

ATCC number: CCL-222
Organism: *Homo sapiens*
Source: Epithelial
Organ: Colon
   - Tumour stage: Dukes’type D
   - Derived from metastatic site: Ascites
Disease: Colorectal adenocarcinoma
Age: 70 years adult
Gender: Male
Ethnicity: Caucasian
This line was isolated in 1975 by T. U. Semple, *et al* from ascitic fluid of a 70 year old caucasian male with carcinoma of the colon. The patient had been treated with 5-fluorouracil for 4-6 weeks before removal of the fluid specimen. The cells are CSAp negative. The cells express a 36K Dalton cell surface glycoprotein related to GA733-2 tumour associated antigen.

**INT407**\(^\text{249}\):

![Image](image.png)

**Figure no. 24: INT407, high density.**

ATCC number: CCL-6 (Intestine 407)
Organism: *Homo sapiens*
Source: Epithelial
Organ: Colon
Gender: Female
This line was originally thought to be derived from normal embryonic intestinal tissue, but was subsequently found to have been established via HeLa cell contamination.
1.18 PLANT PROFILE

i. *Biophytum sensitivum* Linn DC

![Image of Biophytum sensitivum]

**Figure no. 25:** *Biophytum sensitivum* a: whole plant; b: flower; c: herbarium submitted at BSI, Pune.

**A. Classification**

Kingdom: Plantae  
Division: Magnoliophyta  
Class: Magnoliopsida  
Order: Oxalidales  
Family: Oxalidaceae (wood-sorrel family)  
Genus: *Biophytum*  
Species: *sensitivum* Linn

**B. Vernacular names:**

English: Sikerpud  
Hindi: Lajjalu  
Sanskrit: Jalapushpa  
Kannada: Hara muni  
Tamil: Nilaccurunki  
Bengali: Jhalai  
Telugu: Attapatti  
Marathi: Lajwanti

**C. Parts used:** Aerial parts

**D. Habitat:** This plant grows normally in the shade of trees and shrubs, in grasslands, open thickets, at low and medium altitudes.

**E. Botanical description:**

*Biophytum sensitivum* is a very small flowering plant. It is a slender erect annual herb with a rosette of leaves on the top of the stem, with 8-10 leaflets on either sides spreading out from a common base. Each leaflet measures 4-5 mm and the total length of the leaf is less than five cm. Each plant produces five to ten small flowers.
with yellow petals. Each leaflet is up to 1.5 cm long and has an ability to fold together which is exhibited by a lot of members in this family. Fruits are ellipsoid capsules. Seeds are prominently ridged, transversely striate.

**F. Traditional uses:**

It is used as an anti-inflammatory agent, stimulant and in the treatment of stomach ache, diabetes, asthma, chest complaints, convulsions, cramps and inflammatory tumours. Ash of the plant is mixed with lime juice and given for stomach ache. Leaves and roots are styptic; decoction of leaves is given for diabetes, asthma and phthisis. It contains phytoconstituents that inhibit COX-1 and COX-2 catalyzed prostaglandin synthesis.
ii. *Costus speciosus* (Koen) J.E.Smith

![Figure no. 26: Costus speciosus a: whole plant; b: flower; c: herbarium submitted at BSI, Pune.]

A. Classification: 

Kingdom: Plantae  
Division: Magnoliophyta / Angiosperms  
Class: Liliopsida  
Order: Zingiberales  
Family: Costaceae  
Genus: Costus  
Species: C. speciosus

B. Vernacular names: 

English: Crepe ginger, Malay ginger  
Sanskrit: Pushkarmula, kustha  
Tamil: Kostam  
Telugu: Kevukinna  
Hindi: Keukand  
Kannada: Changalakoshta  
Bengali: Keumal/kemuk  
Marathi: Pushkarmula

C. Parts used: Aerial parts 

D. Habitat: It is found in low lying areas in forests and shrubberies.

E. Botanical description: 

It is a small annual succulent herb which is found in shady places. Stem and leaves are juicy. It has only one row of spirally arranged leaves and spirally arranged stems. Stems grow upto a height of 1-3 metres. Leaves are mostly big and about 12-35 cm long. They are mostly without petiole. Flowers are white in colour, with red bracts and black seeds.
F. Traditional uses:
The rhizome is useful in treating burning sensation, flatulence, constipation, helminthiasis, leprosy, skin diseases, fever, hiccough, asthma, bronchitis, inflammation and anemia. It is used to make hormones and contraceptives (semisynthetic). Alkaloids of *Costus speciosus* have been shown to possess anticholinesterase activity in both *in vitro* and *in vivo* studies. Peroral administration of aqueous extract of *Costus speciosus* has significant hypoglycemic effect when the juice is fed with a simultaneous glucose load. Roots are used as tonic and also in fever.
iii. *Gendarussa vulgaris* Nees

![Plant](image1) ![Flower](image2) ![Herbarium](image3)

Figure no. 27: *Gendarussa vulgaris* a: whole plant; b: flower; c: herbarium submitted at BSI, Pune.

A. Classification:\textsuperscript{254, 255}

- **Kingdom**: Plantae
- **Division**: Magnoliophyta
- **Class**: Magnoliopsida
- **Order**: Lamiales
- **Family**: Acanthaceae
- **Genus**: *Gendarussa*
- **Species**: *vulgaris*

B. Vernacular names:

- **English**: Gandarusa, Warer willow
- **Hindi**: Nili nargandi,
- **Sanskrit**: Kasanah, Bhutakeshi
- **Kannada**: Karalakkigidde
- **Tamil**: Karunochi, Vadaikkutti
- **Bengali**: Jagatmadan
- **Telugu**: Addasaramu, Gandharasamu
- **Marathi**: Bakas, Kalaadulsas

C. Parts used: Aerial parts

D. Habitat: It is seen in cultivated beds.

E. Botanical description:

It is a deciduous shade loving, quick growing, evergreen scented shrub. The flowers are hermaphrodite. The plant grows in light (sandy), medium (loamy) and heavy (clay) soils which may be acid, neutral and basic.

F. Traditional uses:

The plant is used in traditional medicinal practice for chronic rheumatism, inflammations, bronchitis, vaginal discharges, dyspepsia, eye diseases and fever. The leaf is antispasmodic, carminative, diaphoretic. The root is anodyne, diuretic and laxative. The root bark is emetic.
iv. *Haplanthus tentaculatus*

![Haplanthus tentaculatus](image)

Figure no. 28: *Haplanthus tentaculatus* a: whole plant; b: flower; c: herbarium submitted at BSI, Pune.

A. **Classification**:  
- **Kingdom**: Plantae  
- **Division**: Magnoliophyta  
- **Class**: Magnoliopsida  
- **Order**: Scrophulariales  
- **Family**: Acanthaceae  
- **Genus**: *Haplanthus*  
- **Species**: *tentaculatus*

B. **Vernacular names:**  
- English: Tentacled Haplanthodes, Nilgiri Haplanthodes  
- Marathi: Neela jakara  
- Konkani: Vhandlem kalem kiraytem

C. **Parts used:** Aerial parts

D. **Habitat:** It grows in moist and evergreen forests.

E. **Botanical description:**  
It is an erect annual herb, 1-2ft tall, with a stout stem and ascending densely hairy branches. Leaves can be opposite or alternate. They are ovate, 5-10 cm long, hairy, with a wavy margin and base is either rounded or asymmetrically heart-shaped. Leaf stalks are winged, 2-4 cm long. Stalkless flowers are pale violet, 5-6 mm, in two rows with 5-15 cm long bristly spikes. Flowers are funnel shaped, with five petals, hairy outside, streaked with browish purple lines.

F. **Traditional uses:** Decoction of plant is given in fever.
v. *Indoneesiella echioides* (L) Sreem

Figure no. 29: *Indoneesiella echioides* a: whole plant; b: flower; c: herbarium submitted at BSI, Pune.

A. Classification:
- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Scrophulariales
- Family: Acanthaceae
- Genus: *Indoneesiella*
- Species: *echioides*

B. Vernacular names:
- English: False water willow
- Gujarati: Kalukariyatun
- Tamil: Gopuram tangi
- Oriya: Lavalata
- Malayalam: Pitumba
- Marathi: Rannchimani

C. Parts used: Aerial parts

D. Habitat: It is found widely growing.

E. Botanical description:
False waterwillow is an annual herb with very hairy stems growing up to 45 cm tall and is branched from the base. Leaves are oblong, up to 7.5 cm long, 2.4 cm wide, or sometimes oblong-spade-shaped, narrowed at the base. Leaves are hairy on both sides. Flowers are borne in spike-like racemes, up to 2 cm long. The stalk carrying the raceme is densely hairy. Flowers are erect. Sepal tube is 2 mm long, with thread-like sepals up to 9 mm long. Flowers have a 4 mm long tube, opening into two lips. Upper
lip is oblong, up to 5.5 x 2 mm, 2-lobed above. Lower lip is up to 7 mm long, with 3 oblong-lance shaped lobes, marked with purple. Stamen filaments are flattened.

F. Traditional uses:
Leaf juice is very bitter to taste and is administered for its anthelmintic activity. Leaf powder is consumed along with rice water for eczema and snake bite. It is also used as a remedy for fevers.
vi. **Lepidagathus cuspidata** Nees

![Figure no. 30: Lepidagathus cuspidata](image)

**Figure no. 30:** *Lepidagathus cuspidata* a: whole plant; b: flower; c: herbarium submitted at BSI, Pune.

**A. Classification**:
- **Kingdom**: Plantae
- **Division**: Magnoliophyta
- **Class**: Magnoliopsida
- **Order**: Scrophulariales
- **Family**: Acanthaceae
- **Genus**: *Lepidagathus*
- **Species**: *cuspidata*

**B. Vernacular names:**
- English: Spiny Lepidagathis
- Marathi: Kate adulsa

**C. Parts used**: Aerial parts

**D. Habitat**: It is found in open hill sides.

**E. Botanical description:**
Spiny Lepidagathis is an erect spiny shrub, up to 1-3 ft high. Stems and branches are quadrangular. Leaves are oppositely arranged and oblong-lance like, about 5-8 cm long. The base tapers into a leaf stalk 1-2 cm long. Upper leaves are smaller, stalkless and with spiny tips. Stalkless flowers appear in spikes 5-10 cm long, at the end of branches. Bracts are 1 cm long and pointed. Flowers are creamish white and 2-lipped. Throat and the upper lip are marked with brownish purple lines. Lower lip is 3-lobed.

**F. Traditional uses**: Root powder is used with milk in fever; flowers are a source of bee-forage.

![Image of Mukia maderaspatana](image_url)

**Figure no. 31:** *Mukia maderaspatana* a: whole plant; b: flower; c: herbarium submitted at BSI, Pune.

### A. Classification

Kingdom: Plantae  
Division: Magnoliophyta  
Class: Magnoliopsida  
Order: Cucurbitales  
Family: Cucurbitaceae  
Genus: *Mukia*  
Species: *maderaspatana*

### B. Vernacular names:

- **English:** Rough bryony  
- **Hindi:** Aganaki, Agumaki  
- **Sanskrit:** Krтарandhrah, Musimusikkay, Trikosaki  
- **Kannada:** Chitrati  
- **Tamil:** Musumuskai, Nagilangiai  
- **Manipuri:** Lam-thambi  
- **Telugu:** Musumusukaya, Potti budamu  
- **Marathi:** Bilavi

### C. Parts used:

Aerial parts

### D. Habitat:

It is found in damp places in woodland, grassland and riverine margins.

### E. Botanical description:

It is a slender, scadentor, prostrate annual herb. Stems, leaves and tendrils have rough, whitish hairs. The leaves are variable, broadly ovate to triangular or palmately 3-5 lobed, with serrate margin. Flowers and fruits are found in small axillary clusters.

### F. Traditional uses:

It is used for the treatment of dog-bite, vertigo, jaundice, toothache and biliousness. It is also used as an expectorant. The tender shoots and bitter leaves are used as gentle aperients. It has been shown to exert hepatoprotective, antioxidant, anti-inflammatory, anti-arthritic, immunomodulatory, anti-microbial and anti-platelet activities. Leaves have constituents that decrease the blood pressure and lower lipid levels.
viii. *Pentatropis nivalis* (Gmel.) D.V. Field & S.R.I. Woods

![Figure 32: Pentatropis nivalis, a: whole plant; b: flower; c: herbarium submitted at BSI, Pune.](image)

A. Classification:
- **Kingdom**: Plantae
- **Division**: Magnoliophyta
- **Class**: Magnoliopsida
- **Order**: Gentianales
- **Family**: Asclepiadaceae
- **Genus**: *Pentatropis*
- **Species**: *nivalis*

B. Parts used: Aerial parts

C. Habitat: It is found in the desert region.

D. Botanical description:

The plant is ascending and twining. Shoots are herbaceous, sparsely pubescent along a single line with 0.18-0.2 mm long glabrescent trichomes. Leaves have 2-3 mm long petiole; colleters are absent; leaf blades are herbaceous, 12-27 mm long, 7-9 mm wide, elliptic, basally rounded, apically obtuse, adaxially isolatedly covered with 0.2-0.25 mm long trichomes restricted to the midvein and the margins, abaxially glabrous. Inflorescences are sessile, 2-3-flowered, all flowers open synchronously.

E. Traditional uses: Flowers are used medicinally and the tubers are edible.
ix. *Vanda tessellata* (Roxb.) Hook. ex G.Don

Figure no. 33: *Vanda tessellata* a: whole plant; b: flower; c: herbarium submitted at BSI, Pune.

A. Classification:

Kingdom: Plantae  
Division: Magnoliophyta  
Class: Liliopsida  
Order: Orchidales  
Family: Orchidaceae  
Genus: Vanda  
Species: *tessellata*

B. Vernacular names:

English: Rinco orchid  
Hindi: Rasnanai  
Sanskrit: Rasna, Vrikshakadali

C. Parts used: Aerial parts

D. Habitat: It grows from Uttar Pradesh to West Bengal and extends to the south of India

E. Botanical description:

It is an epiphytic orchid with special whitish grey coloured velamen roots for the absorption of water. The leaves are thickly coriaceous, recurved, complicate obtusely keeled, and 15-20 cm. x 1.3-2.0 cm in size. The flowers are in 6-10-flowered racemes. Sepals are tessellated with brown lines and white margins. Petals are yellow with brown lines and yellow margins, shorter than the sepals. Lips are bluish, dotted with purple and side lobes are rising from the mouth of the spur. The spur is straight and conical. The capsules are narrowly clavate-oblong, with acute ribs and a short pedicel.
**F. Traditional uses:** Rasna is bitter in taste, pungent in the post digestive effect and has hot potency. It augments the uterine contractions and is a bronchodilator, digestant and blood purifier. It is used in diseases like gout, rheumatic disorders, asthma, abdominal pain, fever and edema.
Introduction

x. *Vernonia cinereae* (L.) Less.

Figure no. 34: *Vernonia cinereae* a: whole plant; b: flower; c: herbarium submitted at BSI, Pune.

A. Classification:
- Kingdom: Plantae
- Division: Magnoliophyta
- Order: Asterales
- Family: Asteraceae
- Genus: *Vernonia*
- Species: *cinereae*

B. Vernacular names:
- English: Ash-coloured fleabane
- Hindi: Sahadevi
- Malayalam: Puvvamkurunnila

C. Parts used: Aerial parts

D. Habitat: It is widely distributed in grassland area.

E. Botanical description:
It is an erect slender, rarely branching annual herb, 12-75 cm in height. The stems are ribbed, finely pilose and glandular. The leaves are alternate, lower ones being perioled while the upper ones are reduced and sessile, oval or broadest about or above the middle and tapering to each end, shallowly toothed. They measure between 2-6 cm long; more or less densely and finely hairy. The heads are small pedunculated, in open, loose corymbs, about 7 mm long, and 2.5 mm in diameter. The flowers are all tubular, bright pink, purple or white. The pappus bristles while, dentate, measuring 3-5 mm long. The achenes are rounded, nearly ribless and measure about 1.5 mm long.
F. Traditional uses:
The whole plant is considered to promote perspiration in febrile condition (diaphoretic). The plant is anthelmintic, antibacterial, antiviral, antifungal, anti-inflammatory, diuretic and stomachic. The roots are useful in diarrhoea, cough, inflammations, skin diseases, leprosy, renal and vesicle calculi. The leaves are useful in humid herpes, eczema, ring worm, guinea worms, and elephantiasis. The flowers are used in conjunctivitis and fever. The seeds are useful in roundworms, threadworms, cough, flatulence, leucoderma, psoriasis, chronic skin disease, antiflatulent, antispasmodic. It is used as a specific herb for leucorrhoea, dysuria, spasm of bladder, strangury and for haematological disorders, as a blood purifier and styptic. It is also used in asthma.