CHAPTER- 2

REVIEW OF LITERATURE
# CHAPTER - 2

## CHAPTER - 2: REVIEW OF LITERATURE

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of the Sub-Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Etiology of Cancer (Neoplasia)</td>
<td>11-16</td>
</tr>
<tr>
<td>2.2</td>
<td>Pathogenesis of Cancer</td>
<td>16-18</td>
</tr>
<tr>
<td>2.3</td>
<td>Cancer Cell &amp; Cell Cycle</td>
<td>18-21</td>
</tr>
<tr>
<td>2.4</td>
<td>Cancer Treatment</td>
<td>21-27</td>
</tr>
<tr>
<td>2.5</td>
<td>Gene Therapy for Cancer</td>
<td>27-28</td>
</tr>
<tr>
<td>2.6</td>
<td>Drawback of Cancer Chemotherapy</td>
<td>28-29</td>
</tr>
<tr>
<td>2.7</td>
<td>Plant Based Anti Cancer Drugs</td>
<td>29-30</td>
</tr>
<tr>
<td>2.8</td>
<td>Review of Herbal Medicinal Plants</td>
<td>30-33</td>
</tr>
<tr>
<td>2.9</td>
<td>Pain</td>
<td>33-36</td>
</tr>
<tr>
<td>2.10</td>
<td>Mechanism of Pain</td>
<td>37-38</td>
</tr>
<tr>
<td>2.11</td>
<td>Pain pathways</td>
<td>39-41</td>
</tr>
<tr>
<td>2.12</td>
<td>Opioid Peptides and Receptors</td>
<td>41-42</td>
</tr>
<tr>
<td>2.13</td>
<td>Met-enkephalin and Leu-enkephalin</td>
<td>42-43</td>
</tr>
<tr>
<td>2.14</td>
<td>Management of pain</td>
<td>43-46</td>
</tr>
<tr>
<td>2.15</td>
<td>Herbal Therapies</td>
<td>46-47</td>
</tr>
<tr>
<td>2.16</td>
<td>Animals Models to Screen Analgesic Activity</td>
<td>47</td>
</tr>
<tr>
<td>2.17</td>
<td>Inflammation</td>
<td>48-54</td>
</tr>
<tr>
<td>2.18</td>
<td>Chronic Inflammation</td>
<td>54-58</td>
</tr>
<tr>
<td>2.19</td>
<td>Animal Models to Screen Anti-inflammatory Activity</td>
<td>58-60</td>
</tr>
<tr>
<td>2.20</td>
<td>Literature Review of <em>Citrus maxima</em> (J.Burm.) Merr</td>
<td>60-63</td>
</tr>
<tr>
<td>2.21</td>
<td>Literature Review of <em>Citrus aurantium</em> Lin.</td>
<td>63-69</td>
</tr>
</tbody>
</table>
CHAPTER -2: REVIEW OF LITERATURE

The terms cancer, malignant neoplasm (neoplasm simply means 'new growth') and malignant tumor are synonymous.\(^2\) A disease in which there is a mass of tissue formed as a result of abnormal, excessive, uncoordinated, autonomous and purposeless proliferation of cells is called neoplasm or tumor. When they are slow growing and localized without causing much difficulty to the host called as a **benign** and when they are proliferate rapidly, spread throughout the body and may eventually cause death of the host called as a **malignant**. Cancer is a common term for all malignant tumors. There are hundreds type of cancers which can affect any part of the body.\(^{15}\)

Both benign and malignant tumors manifest uncontrolled proliferation, but the latter are distinguished by their capacity for dedifferentiation, their invasiveness and their ability to metastasize. The appearance of these abnormal characteristics reflects altered patterns of gene expression in the cancer cells, resulting from genetic mutations.\(^2\)

A tumor is said to be benign when its microscopic and gross characteristics are considered to be relatively innocent, implying that it will remain localized, it cannot spread to other sites, and is amenable to local surgical removal; the patient generally survives. It should be noted, however, that benign tumors can produce more than localized lumps, and sometimes they are responsible for serious disease.\(^{16}\)
Malignant tumors are collectively referred to as cancers, derived from the Latin word for crab—that is, they adhere to any part that they seize in an obstinate manner, similar to a crab’s behavior. Malignant, as applied to a neoplasm, implies that the lesion can invade and destroy adjacent structures and spread to distant sites (metastasize) to cause death. Not all cancers pursue so deadly a course. Some are less aggressive and are treated successfully, but the designation malignant constitutes a red flag.\textsuperscript{16} Other approaches to cancer treatment, based, for example, on our increasing knowledge of the etiology and pathogenesis of cancer.\textsuperscript{2}

2.1 Etiology of Cancer (Neoplasia):

Cancer is caused by both external and internal factors respectively. External factors include; tobacco, chemicals, radiation, and infectious organisms and internal factors include; 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.\textsuperscript{17} The numerous agents capable of producing neoplasia naturally and experimentally are Carcinogenic chemicals, Radiation, Oncogenic viruses, Environmental factor and others. The incidence, geographical distribution and behavior of specific type of cancer are related to multiple factors including exposure to carcinogens, environmental condition (anoxia, oxidative stress), oncogenic viruses, genetic predisposition, sex, age, and race. Among these, exposure to carcinogen is most common.
2.1.1 Exposure of Carcinogens:

Carcinogens are the physical and chemical agents that induce the incidence of cancer in the animal model and human population. Physical agents follow.

**Irradiation:** is the main cause of physical form of cancer in human skin. The actual mechanism behind the X-rays related cancer is chromosomal damage.\(^{18}\)

Ultra –Violet (UV) rays can also cause skin cancer wherever the autonomic recessive disorder and xerodermic conditions are predominant. In some cases, the skin overexposed to direct sunlight might cause skin cancer by DNA repair mechanism. Malignant melanoma and other skin cancers are common in black skinned people but are particularly frequent in fair – skinned cells.

**Asbestos:** The most common form of cancer associated with asbestos exposure is malignant mesothelioma, but the risk of bronchogenic cancer is also significantly elevated. Asbestos fibres are cytotoxic and genotoxic. They have been shown to induce DNA damage, including double-strand breaks, mutations and chromosomal damage.

2.1.2 Chemical Carcinogens:\(^{15,16}\)

Many works have extensively studied the mechanism of action of chemical carcinogens. Some important classes of chemical carcinogens, their reactive forms or sources and their target organs are mentioned below.

- **Polycyclic hydrocarbons:** These are derived from the cigarette
smoke, car exhaust and fumes. Polycyclic hydrocarbons react with base pairs of cellular DNA, inflict changes and finally result in cancer, as in the case of lung cancer.

- **Aromatic amines and azo dyes:** Beta-napthylamine and Benzidine are the main active forms that react with the liver cells and once excreted from liver they reach Urinary Bladder to cause Bladder cancer.

- **Nitrosamine:** Various stored or tinned foods contain nitrites, which are converted into nitrates when ingested. Nitrates further react with amines and cause stomach cancer.

- **Alkylating agents:** Most of the drugs consumed for various ailments have this form that bind with DNA leading to cancer development.

- **Organic toxins:** One of the most important sources is aflatoxin, which is produced by the fungus, “Aspergillus flavus”. Aflatoxin combines with Hepatitis B Virus and causes lung cancer.

### 2.1.3 Viral or Bacterial Infection:

Some cancers can be caused by infection with pathogens. Several cancers which originate from a viral infection are the facts in animals, birds and humans. The main human cancer causing viruses are, human papilloma virus, hepatitis B, hepatitis C virus, Epstein-Barr virus, and human T-lymphotropic virus. The mode of virally-induced tumors can be divided into two, acutely transforming or slowly transforming. In acutely transforming viruses, the virus carries
an overactive oncogene called viral-oncogene (v-onc), and the infected cell is transformed as soon as v-onc is expressed. Whereas, in slowly-transforming viruses, the virus genome is inserted near a proto-oncogene in the host genome. Then, the proto-oncogene is over expressed by viral promoter or other transcription regulation elements. Thus, it induces uncontrolled cell division. Because the site of insertion is not specific to proto-oncogenes and the chance of insertion near any proto-oncogene is low, slowly-transforming viruses will cause tumors much longer after infection than the acutely-transforming viruses.\textsuperscript{15,16}

2.1.4 Hormonal Imbalances:

Excessive cell growth is stimulated by some hormones which can act in a similar manner to non-mutagenic carcinogens. Example, role of hyperestrogenic states in promoting endometrial cancer.\textsuperscript{16}

2.1.5 Immune System Dysfunction:

HIV is associated with a number of malignancies, including Kaposi's sarcoma, non-Hodgkin's lymphoma and HPV-associated malignancies such as anal cancer and cervical cancer. The HIV patients point to the breakdown of immune surveillance as a possible etiology of cancer is mainly due to the increased incidence of malignancies. Certain other immune deficiency states (e.g. common variable immunodeficiency and IgA deficiency) are also associated with increased risk of malignancy.\textsuperscript{15,16}

2.1.6 Heredity:

Most forms of cancer are "sporadic", and have no basis in
heredity. There are, however, a number of recognized syndromes of cancer with a hereditary component, often a defective tumour suppressor allele. Examples: Certain inherited mutations in the genes BRCA1 and BRCA2 - elevated risk of breast cancer and ovarian cancer, tumors of various endocrine organs in multiple endocrine neoplasia, Li-Fraumeni syndrome (osteosarcoma, breast cancer, soft tissue sarcoma, brain tumours) due to mutations of p53, Turcot syndrome (brain tumors and colonic polyposis), Familial adenomatous polyposis - an inherited mutation of the APC gene that leads to early onset of colon carcinoma, Hereditary nonpolyposis colorectal cancer (HNPCC, also known as Lynch syndrome) can include familial cases of colon cancer, uterine cancer, gastric cancer and ovarian cancer, without a preponderance of colon polyps.

2.1.7 Oxidative Stress:

Oxygen in the form of free radicals (molecules, which are highly reactive, they bind and destroy body components) affect the normal cell and cause cancer. In our body, the activity of free radicals are naturally controlled and delimited by another group of chemical compounds called “anti-oxidants” which are present in higher levels in our regularly consumed food materials. The beta-carotene, vitamin C and vitamin E (Tocopherol) are some important antioxidant substances. These are known to be effective against cancer by scavenging antioxidants are strictly balanced. Under certain conditions, the group of free radical is increased above normal that stimulates the normal to become cancerous. The reactive oxygen
species like superoxide anions, hydroxyl radicals and hydrogen peroxide are produced as by-products of oxidative respiration and lipid metabolism. All these are permanent enemies for the integrity of DNA within the cell.

2.1.8 Other Causes:

Excepting the rare transmissions that occur with pregnancies and only a marginal few organ donors, cancer is generally not a transmissible disease. The main reason for this is tissue graft rejection caused by MHC incompatibility. In humans, the immune system uses MHC antigens to differentiate between "self" and "non-self" cells because these antigens are different from person to person. When non-self antigens are encountered, the immune system reacts against the appropriate cell. Such reactions may protect against tumour cell engraftment by eliminating implanted cells.

2.2 Pathogenesis of Cancer

Cancer cells manifest, to varying degrees, four characteristics that distinguish them from normal cells are, uncontrolled proliferation, dedifferentiation & loss of function, invasiveness and metastasis.

A normal cell turns into a cancer cell because of one or more mutations in its DNA, which can be acquired or inherited. A good example is breast cancer; women who inherit a single defective copy of either of the tumor suppressor genes BRCA1 and BRCA2 have a significantly increased risk of developing breast cancer. However, carcinogenesis is a complex multistage process, usually involving
more than one genetic change as well as other, epigenetic factors (hormonal, cocarcinogen and tumor promoter effects, etc.) that do not themselves produce cancer but which increase the likelihood that genetic mutation will result eventually in cancer.²

**Fig. 2.1: Pathogenesis of Cancer**

Cancer cells have uncontrolled proliferation because of changes in growth factors and/or their receptors intracellular signalling pathways, particularly those controlling the cell cycle and apoptosis, telomerase expression, tumor-related angiogenesis. Cancer arises as a result of a series of genetic and epigenetic changes, the main genetic lesions being:

For the activation of proto-oncogenes to oncogenes; Proto-oncogenes are genes that normally control cell division, apoptosis and differentiation, but which can be converted to oncogenes that induce malignant change by viral or carcinogen action.
For the inactivation of tumor suppressor genes, Normal cells contain genes that have the ability to suppress malignant change-termed tumor suppressor genes (antioncogenes) and there is now good evidence that mutations of these genes are involved in many different cancers. The loss of function of tumour suppressor genes can be the critical event in carcinogenesis.\(^2\)

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

### 2.3 Cancer Cell & Cell Cycle

The basic organization of normal cells and cancer cells is similar and is composed of materials. Cancer cells utilize the same nutrients and produce the same waste products as do normal cells. Mostly cancer cells and normal cells contain many of the same enzyme and other proteins, whereas cancer cells often contain an altered array of proteins when compared to their normal counterparts. The step and movement of cells are determined by changes in the organization of microtubules and microfilaments. The dramatic changes in structure and mobility that occur during neoplastic transformation are due to reorganization of the intracellular network of microtubules and microfilaments.\(^20\)

Growth and multiplication of cancer cells and normal cells occur by the same fundamental process, invasive, metastatic and genetically unstable properties of cancer cells are exhibited by various
normal cells at various times during normal growth. Reproduction of chromosomes in cancer cells is highly error prone when compared to the normal cell process. Cancer cells differ from normal cells in four fundamental ways:

1) Uncontrolled proliferation.
2) Loss of contact inhibition to slow growth.
3) Lack of adhesion requirement for growth.
4) Inability to differentiate fully.²⁰

2.3.1 Cell Cycle²¹

Both the cancer and normal cell reproduces in a series of steps known as cell cycle. The cell cycle phases are: Resting (G₀; nothing is happening), G₁ (or gap 1; a growth phase), S (synthesis; the replication of DNA occurs), G₂ (gap 2; another growth phase), and M (mitosis; the actual division from 1 cell into 2).

The processes outlined in the cycle (figure 3) occur inside a cell such as the one shown in Figure. A quiescent cell (in G₀ phase), when stimulated to divide by growth factors, is propelled into G₁ phase and prepares for DNA synthesis. Progress through the cycle is determined by sequential action of the cyclin/cdk complexes—depicted here by coloured arrows, the arrows being given the names of the relevant cyclins: D, E, A and B. The cdks (cyclin-dependent kinases) are given next to the relevant cyclins. The thickness of each arrow represents the intensity of action of the cdk at that point in the cycle. The activity of the cdks is regulated by cdk inhibitors.

If there is DNA damage, the products of the tumour suppressor
gene p53 stop the cycle at check point 1, allowing for repair. If repair fails, apoptosis is initiated. The state of the chromosomes is shown in each G phase as a single pair in G1, and each duplicated and forming two daughter chromatids in G2. Some changes that occur during mitosis (metaphase, anaphase) are shown in a subsidiary circle. After the mitotic division, the daughter cells may enter G1 or G0 phase. There are eight main groups of cyclins. Those important in the control of the cell cycle are cyclins A, B, D and E. Each cyclin is associated with and activates particular cdk(s). Cyclin A activates cdks 1 and 2; cyclin B, cdk 1; cyclin D, cdks 4 and 6; and cyclin E, cdk 2.

**Fig. 2.2: Cell Cycle**

Precise timing of each activity is essential, and many cycle proteins are degraded after they have carried out their functions. The
activity of these cyclin/cdk complexes is modulated by various negative regulatory forces (considered below), most of which act at one or other of the two check points.

2.3.2 **Many morphological, biological and biochemical changes are seen in cancer cells.**

Morphological changes in cancer cells: Cancer cells are usually rounded shape larger than the normal cell. Cancer cells show nuclear and cellular pleomorphism, hyper chromatist and altered nuclear, Cancer cell often grow over one another and from multilayer, Cancer cell have least adhesion capacity and grow without attachment to the surface *in vitro*. Large nucleoli are present, Nuclear chromatin is often coarsely clumped and distributed along the nuclear membrane.

Biochemical Change in cancer cells: Increase the turnover of the nucleic acid, DNA and RNA. Enhanced glycolysis in both aerobic and anaerobic, Alteration in cell permeability and cell surface change, Increased activity of ribonucleotide reductase, Secrete certain protein kinase, Cancer cells show appearance of new antigen and loss of certain normal antigen, Exhibit inappropriate synthesis of certain hormones and growth factor.

2.4 **Cancer Treatment:**

Many options for cancer exist including: chemotherapy, radiation therapy, surgery, immunotherapy, monoclonal antibody therapy and other methods. Which are used depends upon the location and grade of the tumour and the stage of the disease, as well as the general state of a person’s health.
2.4.1 Surgery:

Surgery has a pivotal role in the management of the cancer. Surgery offers the greatest chance for cure for many types of cancer, especially those that have not spread to other parts of the body. In some cases, a margin of healthy tissue and nearby lymph nodes may also be removed to try to prevent spread of the cancer. Surgery may be used to treat cancer when it is first diagnosed. When the cancer cannot be cured, surgery is sometimes used to help control the disease and alleviate symptoms.

2.4.2 Radio Therapy:

Radio therapy means treatment of cancer with ionizing radiation emitted from the decay of radioactive isotope or high energy of beam of radiation like X – ray. It may be used before surgery to shrink the tumour or after surgery to destroy any remaining cancer cells. In external radiation therapy, a machine beams the rays at the cancer. In some cases, an implant containing a radioactive substance may be inserted into the tumour to deliver a higher dose of radiation than is possible externally. These can be delivered by;

2.4.2.1 Teletherapy:

Radiation from a distance by a linear accelerator.

2.4.2.2 Brachytherapy:

Direct application of radioactive source on tumor by delivering very high localize dose. Intravenous injection of radio isotope: - such as I131 for thyroid cancer and St89 for bone metastasis from prostate cancer. Ionizing radiation used are Photons (x-rays and gamma rays),
which are most widely used and Particle radiation (electrons, protons, neutrons, alpha particles, and beta particles). Some types of ionizing radiation have more energy than others. The higher the energy, the more deeply the radiation can penetrate (get into) the tissues. The way a certain type of radiation behaves is important in planning radiation treatments.

2.4.3 Chemotherapy:

Chemotherapy, in its most general sense, is the treatment of disease by chemicals. Most anticancer drugs are antiproliferative-most damage DNA and thereby initiate apoptosis. They also affect rapidly dividing normal cells and are thus likely to depress bone marrow, impair healing and depress growth. Most cause nausea, vomiting, sterility, hair loss and teratogenicity.

a. Alkylating agents:

Alkylating agents are so named because of their ability to add alkyl groups to many electronegative groups under conditions present in cells. Cisplatin and carboplatin, as well as oxaliplatin, are alkylating agents. They impair cell function by forming covalent bonds with the amino, carboxyl, sulfhydryl, and phosphate groups in biologically important molecules. Other agents are mechlorethamine, cyclophosphamide, chlorambucil, ifosfamide. They work by chemically modifying a cell's DNA.

b. Anti-metabolites:

Anti-metabolites masquerade as purine (azathioprine, mercaptopurine) or pyrimidines which become the building blocks of
DNA. They prevent these substances from becoming incorporated into DNA during the "S" phase (of the cell cycle), stopping normal development and division. They also affect RNA synthesis. Due to their efficiency, these drugs are the most widely used cytostatics.

**c. Plant Alkaloids and Terpenoids:**

These alkaloids are derived from plants and block cell division by preventing microtubule function. Microtubules are vital for cell division, and, without them, cell division cannot occur. The main examples are vinca alkaloids and taxanes.
Fig. 2.3: Summary of the Main Site of Action of Cytotoxic Agents

a. Vinca Alkaloids:

Vinca alkaloids bind to specific sites on tubulin, inhibiting the assembly of tubulin into microtubules (M phase of the cell cycle). They are derived from the Madagascar periwinkle, *Catharanthus roseus*. 
(formerly known as *Vinca rosea*). The vinca alkaloids include: vincristine, vinblastine and vinorelbine.

**b. Podophyllotoxin:**

Podophyllotoxin is a plant-derived compound which is said to help with digestion as well as used to produce two other cytostatic drugs, etoposide and teniposide. They prevent the cell from entering the G1 phase (the start of DNA replication) and the replication of DNA (the S phase). The exact mechanism of its action is not yet known. The substance has been primarily obtained from the American Mayapple (*Podophyllum peltatum*). Recently it has been discovered that a rare Himalayan Mayapple (*Podophyllum hexandrum*) contains it in a much greater quantity, but, as the plant is endangered, its supply is limited. Studies have been conducted to isolate the genes involved in the substance’s production, so that it could be obtained recombinantly.

**c. Taxanes:**

The prototype taxane is the natural product paclitaxel, originally known as Taxol and first derived from the bark of the Pacific Yew tree. Docetaxel is a semi-synthetic analogue of paclitaxel. Taxanes enhance stability of microtubules, preventing the separation of chromosomes during anaphase.

**d. Topoisomerase Inhibitors:**

Topoisomerases are essential enzymes that maintain the topology of DNA. Inhibition of type I or type II topoisomerases interferes with both transcription and replication of DNA by upsetting proper DNA supercoiling. Some type I topoisomerase inhibitors
include *camptothecins*: irinotecan and topotecan.

Examples of type II inhibitors include amsacrine, etoposide, etoposide phosphate, and teniposide. These are semisynthetic derivatives of epipodophyllotoxins, alkaloids naturally occurring in the root of American Mayapple (*Podophyllum peltatum*).

e. **Antitumor Antibiotics:**

These include the immunosuppressant dactinomycin (which is used in kidney transplantations), doxorubicin, epirubicin, bleomycin and others.

**2.5 Gene Therapy for Cancer:**

Oncogenes are the gene responsible for the causation of the cancer. Dominantly acting oncogene can be targeted in the antisense technology using antisense transgene or oligonucleotide. These are generally binds to the mRNA and block protein biosynthesis (translation). This therapy is used in treatment of myeloid leukemia, cancer of prostate gland and brain tumour. Some approaches include

- Restoring 'protective' proteins such as the tumor suppressor gene p53.
- Inactivating oncogene expression (e.g. by using a retroviral vector bearing an antisense transcript RNA to the k-ras oncogene).
- Delivering a gene to malignant cells that renders them sensitive to drugs (e.g. thymidylate kinase, which activates ganciclovir).
- Delivery of proteins to healthy host cells in order to protect
them (e.g. addition of the multidrug resistance channel to bone marrow cells *ex-vivo*, thereby rendering them resistant to drugs used in chemotherapy).

- Tagging cancer cells with genes expressing proteins that render malignant cells more visible to the immune system (e.g. for antigens such as HLA-B7 or cytokines such as granulocyte macrophage colony-stimulating factor and interleukin-2).

### 2.6 Drawback of Cancer Chemotherapy

Though anticancer drugs prolong lives of cancer patients and cure a substantial number, many of these patients develop secondary cancers, some of which are more severe than the primary cancer. Most of the conventional anticancer drugs are toxic but are considered acceptable only because truly safe drugs are unavailable. Due to unavailability of safer and more specific anticancer drugs, the present therapy is considered inevitable. Highly toxic compounds such as doxorubicin, cisplatin, and cyclophosphamide are today’s first line anticancer drugs because we don’t have safer and more effective drugs. As there is no other option except to compromise with side effects. The complications of chemotherapy can be of three types – early, intermediate and late. Early complications (within hours) like, nausea, vomiting, fever and hypersensitivity reactions. Intermediate complications (within days) such as, stomatitis, diarrhoea, alopecia, peripheral neuropathy and bone marrow depression. Late complications (with in few months) manifested as, injury to vital organs, endocrinial changes, teratogenic
effects and physiological effects.\textsuperscript{1} Hence, search for new antitumour agents with high chemotherapeutic value to fight against cancer is a medical priority. Therefore, present study aims to explore the anticancer potential of the selected plant.\textsuperscript{21}

2.7 Plant Based Anti Cancer Drugs

Herbal drugs constitute a major share of all the officially recognised systems of health in India viz. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy, except Allopathy.

More than 70\% of India"s 1.1 billion populations still use these non-allopathic systems of medicine. Currently, there is no separate category of herbal drugs or dietary supplements, as per the Indian Drugs Act. Significant basic and clinical research has been carried out on the medicinal plants and their formulations, with the state-of-the-art methods in a number of Institutes/Universities.\textsuperscript{26}

Plants have a long history of use in the treatment of cancer.\textsuperscript{27} The search for anti-cancer agents from plant sources started in the late 1950, with the discovery and development of the vinca alkaloids, (vinblastine and vincristine) and isolation of cytotoxic podophyllotoxins. As a result, the United States National Cancer Institute (NCI) initiated an extensive plant collection program in 1960. This led to the discovery of many other compounds such as taxanes, camptothecins and combrestatins,\textsuperscript{28} paclitaxel (Taxol), vinorelbine (Navelbine), teniposide (Vumon) and various water-soluble analogs of camptothecin (e.g., Hycamtim) which are being used in cancer
treatment with varied degrees of success. Moreover, plant-based drugs are cheap, locally available, and free from severe side effects.

Over 60% of currently used anti-cancer agents are derived in one way or another from natural sources, including plants, marine organisms, and microorganisms.\textsuperscript{29, 30}

\textbf{2.8 Review of Herbal Medicinal Plants}

Every year about 8, 50,000 new cancer cases being diagnosed, India resulting about 5,80,000 cancer-related death every year. India had the highest number of the oral and throat cancer cases in the world. Every third oral cancer patient in the world is from India. In males, oral, lungs, and stomach cancers was the three most common causes of cancer incidence and death whereas in females, cervical, breast, and oral cancers were the three main causes of cancer-related illnesses and death. Overall cervical cancer was the number one cause of cancer death in India. That was really unfortunate as cervical cancer can be easily prevented and also relatively easy to diagnose and treat at an early stage. Compared to developed countries overall there were less cancer cases in India but that could be due to under diagnosis and under reporting. At the same time, regional, ethnic, dietary, and socio-economic factors might also result in differences in the cancer susceptibilities and the incidence. Also, cancer was mainly a disease of old ages. Worldwide median age at diagnosis was about 60 years. Average life span was about 58 yrs in India compared to 75 yrs in the developed world.

Plant materials were used for the treatment of malignant
diseases for centuries. Recent phytochemical examination of plants which have a suitable history of use in folklore for the treatment of cancer had induced often resulted in the isolation of principles with antitumour activity. An intensive survey of plants, micro organism and marine animals for antitumour activity began in the later 1950s mainly because the United States National Cancer Institute (NCI) instigated and fund a major screening programme. Random selection screening programme was adopted, since novel compounds may be found anywhere from plant or animal kingdom.

Soybean phytochemicals such as genistein (4',5,7-tribydroxy isoflavone) inhibit the growth of transplantable human prostate carcinoma. Epidemiological studies have consistently shown that regular consumption of fruits and vegetables strongly associated with reduced risk of developing chronic diseases such as cancer as the phytochemical extracts from it exhibit strong antioxidant activity. Andrographolide the potential cancer therapeutic agent isolated from Andrographis paniculata 31-33.

**Table. No. 2.1: Indian Medicinal Plants Used for the Treatment of Cancer**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the Plant</th>
<th>Family</th>
<th>Parts Used</th>
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<tr>
<td>1.</td>
<td><em>Calotrophis gigantea</em></td>
<td>Asclepiadaceae</td>
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<td>Fabaceae</td>
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<tr>
<td>3.</td>
<td><em>Butea monosperma</em></td>
<td>Fabaceae</td>
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<td>4.</td>
<td><em>Bauhinia variegata</em></td>
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<td><em>Bacopa monnieri</em></td>
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<td>Cassia auriculata</td>
<td>Caesalpinaceae</td>
<td>Root</td>
</tr>
<tr>
<td>14.</td>
<td>Cassia senna</td>
<td>Caesalpinaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>15.</td>
<td>Catunaregum spinosa</td>
<td>Rubiaceae</td>
<td>Bark/Fruit</td>
</tr>
<tr>
<td>16.</td>
<td>Citrullus colocynthis</td>
<td>Cucurbitaceae</td>
<td>Root</td>
</tr>
<tr>
<td>17.</td>
<td>Citrus medica</td>
<td>Rutaceae</td>
<td>Root</td>
</tr>
<tr>
<td>18.</td>
<td>Cissus quadrangularis</td>
<td>Vitaceae</td>
<td>Whole plant</td>
</tr>
<tr>
<td>19.</td>
<td>Clerodendrum serratum</td>
<td>Verbanaceae</td>
<td>Root</td>
</tr>
<tr>
<td>20.</td>
<td>Clerodendrum viscosum</td>
<td>Verbanaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>21.</td>
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<td>Amaryllidaceae</td>
<td>Bulb</td>
</tr>
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<td>22.</td>
<td>Daucus carota</td>
<td>Apiaceae</td>
<td>Root</td>
</tr>
<tr>
<td>23.</td>
<td>Embelia ribes</td>
<td>Myrsinaceae</td>
<td>Fruit</td>
</tr>
<tr>
<td>24.</td>
<td>Flacourtia jangomos</td>
<td>Flacourtiaceae</td>
<td>Bark/Leaf</td>
</tr>
<tr>
<td>25.</td>
<td>Jatropha curcas</td>
<td>Euphorbiaceae</td>
<td>Leaves, seed, oils</td>
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<td>26.</td>
<td>Kaempferia galanga</td>
<td>Zingiberaceae</td>
<td>Rhizome</td>
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<td>27.</td>
<td>Kaempferia rotunda</td>
<td>Zingiberaceae</td>
<td>Tubers</td>
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<td>Lanata camara</td>
<td>Verbanaceae</td>
<td>Whole plant</td>
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<td>29.</td>
<td>Lens culinaris medikus</td>
<td>Fabaceae</td>
<td>Seed</td>
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<td>Fabaceae</td>
<td>Seed</td>
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<td>Mimosaceae</td>
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<td>Nicotiana tabacum</td>
<td>Solanaceae</td>
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<td>Operculina turpethum</td>
<td>Convolvulaceae</td>
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<td>35.</td>
<td>Rhinacanthus nasuta</td>
<td>Acanthaceae</td>
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</tr>
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<td>No.</td>
<td>Species</td>
<td>Family</td>
<td>Parts</td>
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<td>-----</td>
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<td>-----------------</td>
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<tr>
<td>36.</td>
<td><em>Salvadora persica</em></td>
<td>Salvadoraceae</td>
<td>Bark, Leaf, Shoot, Fruit</td>
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<tr>
<td>37.</td>
<td><em>Symplocus cochinchinensis</em></td>
<td>Symplocaceae</td>
<td>Bark</td>
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<td>38.</td>
<td><em>Tylopora indica</em></td>
<td>Asclepiadaceae</td>
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<td>39.</td>
<td><em>Vernonia cinerea</em></td>
<td>Asteraceae</td>
<td>Whole plant</td>
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<td>40.</td>
<td><em>Vitex trifolia</em></td>
<td>Verbanaceae</td>
<td>Leaf</td>
</tr>
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<td>41.</td>
<td><em>Zanthoxylum armatum</em></td>
<td>Rutaceae</td>
<td>Bark, Fruit</td>
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<tr>
<td>42.</td>
<td><em>Xanthium strumarium</em></td>
<td>Compositae</td>
<td>Root</td>
</tr>
</tbody>
</table>

### 2.9 Pain:

According to the International Association for the Study of Pain, pain is “an unpleasant sensory and emotional experience associated with actual and potential tissue damage." Sometimes, pain is hard to describe. In fact, it can present differently for each person, which is why people perceive and tolerate pain in different ways. The ability to cope with pain differs markedly from person to person. Some people can tolerate severe pain without complaint, where others react strongly to it (by crying or complaining) even though the pain may not be intense. This is why, in most cases, the reaction to pain is not always a reliable indicator of its real intensity. The axiom “No one ever died from pain” is clearly incorrect, given the modern recognition that unrelieved pain increases cardiac work, increases metabolic rate, interferes with blood clotting, leads to water retention, lowers oxygen levels, impairs wound healing, alters immune function, interferes with sleep, and creates negative emotions. Unrelieved pain can, for
example, delay the return of normal gastric and bowel function in postoperative patients.\textsuperscript{41}

2.9.1 Pain can be classified upon following basis:-

- Location of the pain
- Duration of the pain
- Underlying causes of pain
- Intensity of the pain

2.9.1.1 Location of Pain

Pain is often classified by body location. Two overlapping schemes relate the pain to the specific anatomy and/or body system thought to be involved. The anatomic pain classification system identifies sites of pain as viewed from a regional perspective (eg, lower back pain, headache, pelvic pain). In contrast, the body system pain classification method focuses on classical body systems (eg, musculoskeletal, neurologic, vascular).\textsuperscript{42}

2.9.1.2 Duration of Pain

The duration of the pain process is the most obvious distinction that can be made when classifying pain symptoms. Conventionally, acute pain is limited to pain of less than 30 days’ duration, whereas chronic pain persists for more than 6 months.\textsuperscript{43,44}

2.9.1.3 Underlying cause of the pain

a. Somatic Pain

Somatic pain is referred to as musculoskeletal pain. It is inferred to be related to ongoing activation of nociceptors that innervate somatic structures, such as bone, joint, muscle and
connective tissues, is termed “somatic pain”. It is found in tissue such as skin and muscles as well as in joints, bones and ligaments. Somatic pain is often characterized as a sharp pain localized in a specific area of injury. Swelling, cramping and bleeding may exist with somatic pain. It is generally peripheral. The pain that result from somatic processes are constant, gnawing in character. This pain is recognized by identification of a lesion and characteristics that typically include a well localized site and an experience described as aching, squeezing, stabbing, or throbbing. Arthritis and metastatic bone pain are examples of somatic pain.45

b. Visceral Pain

Visceral pain is a type of nociceptive pain located within the main body cavity due to injury or illness to an internal organ. Visceral pain is often described as generalized aching or squeezing. It sends signals to the spinal cord and brain when damage is detected. It is caused by compression in and around the organs, or by stretching of the abdominal cavity. Sometimes visceral pain may radiate to other areas in the body, making it even harder to pinpoint its exact location. The three main centers for visceral pain are the thorax, abdomen and pelvis. The pain receptors in the visceral cavities respond to stretching, swelling and oxygen deprivation. Visceral pain may radiate to other locations in the back and chest.46

c. Neuropathic Pain

Neuropathic pain is usually caused by nerve damage such as that resulting from nerve compression or inflammation or from
diabetes. Neuropathic pain is characteristic, for example Trigeminal neuralgia, postherpetic neuralgia, and certain types of back and limb injuries. This type of pain is severe and usually described as burning or tingling.  

2.9.1.4 Intensity of Pain

The intensity of pain offers, perhaps, the least desirable system for classifying pain, because intensity varies for most patients over time and is uniquely subjective. A patient might rate the experience of pain resulting from some pathologic condition as a 10, whereas another patient with the same pathology might describe the intensity of pain only as a 5, both using a 0 to 10 scale (with 0 signifying no pain at all and 10 representing the worse pain imaginable) (Fig 2.4). Whereas non–cancer related pain is often rated along a continuum (i.e., from mild, to moderate, to severe), the words “incapacitating,” “overwhelming,” and “soul stealing” frequently become necessary qualifiers for cancer pain.  

![Pain rating scale](image)

**Fig. 2.4: Illustration of a Pain-rating Scale for Classifying the Intensity of Pain**
2.10 Mechanism of Pain:

2.10.1 Processing of Pain Signals.

When our skin is damaged, nerve cell endings in the damaged area initiate a sensation of pain. These nerve cell endings are called nociceptors. Nociceptors are relatively unspecialized cell endings or “free endings” depending upon mylineation of these free fibers they are further classified into type A-Fibers and type C fibers.

**C-Fibers** are non-myelinated fibers that conduct in the range of 0.5 m/s to 2 m/s and transmit noxious information from a variety of modalities including mechanical, thermal, and chemical stimuli—for this reason, they are termed C-polymodal nociceptors.

**A-Fibers** are thinly myelinated fibers which conduct in the range of 2 m/s to 20 m/s. All fibers respond to high intensity mechanical stimulation and are therefore termed high threshold mechanoreceptors. Some, but not all fibers also respond to thermal stimuli—the latter are termed mechano-thermal receptors. **A-fibers** are further subdivided into α, β, γ and δ fibers.

![Myelinated and Unmyelinated Fibers](image)
**A-delta group** fibers are myelinated which allows them to conduct faster action potentials. Their function explains why their speed is necessary as they respond primarily to sharp pressure or heat. When touching a hot pan or waving your finger over a flame, it would probably be detrimental to have a slower signaling system rather than a faster one. The slower **C fiber group** of unmyelinated axons responds to intense stimulation to: pressure, heat or cold or noxious chemicals. This kind of pain is the long lasting pain we feel after damage has already been done, such as the long lasting burn related to sunburn. If you recall the last time you had a painful experience, such as a deep gash or cut, you may remember a sharp burst of pain followed by a throbbing sensation. These two stages reflect the first set of signals from the **A-delta group** followed by the second onset of the **C fiber group** as shown by the (Fig 2.6).\(^{50}\)

![Fig. 2.6: Stages of pain](image)
2.11 Pain pathways:

Exposure to a noxious stimulus activates nociceptors on the peripheral free nerve endings of primary afferent neurons. The cell bodies of these neurons sit alongside the spinal cord in the dorsal root ganglia and send one axon to the periphery and one to the dorsal horn of the spinal cord. With noxious stimulation substance-p, Glutamate, and other excitatory neurotransmitters are released.

**Glutamate** – this seems to be the dominant neurotransmitter when the threshold to pain is first crossed, and is associated with acute pain.

**Substance P** – this is a peptide that contains 11 amino acids and is released by C fibers. It is generally associated with intense, persistent, chronic pain, and used to relay pain messages leading to the spinal cord and brain. These neurotransmitters are released from the central terminations of the primary afferent fibers onto neurons of the spinal cord. Many of these terminals synapse directly on spinothalamic tract neurons in the dorsal horn, which send long fibers up the contralateral side of the spinal cord to transmit pain impulses via ascending pain pathways to the medulla, midbrain, thalamus, limbic structures and cortex.
Fig. 2.7: Neurophysiology of incoming pain. Sensation from peripheral receptors travels along specific pain nerves, and is modulated throughout the spinal cord and brain.

The primary afferent fibers, transmitting nociceptive information are Aδ and c fibers. Spinal reflexes activated by these fibers can lead to withdrawal from a noxious stimulus before pain is perceived by higher structures. Ascending pain pathways consist of two main anatomical-functional projections; the sensory-discriminative component to the cerebral cortex and the motivational-affective component, to the limbic cortex. Projections to the sensory cortex alert an individual to the presence and anatomic location of pain. The activation of spinothalamic neurons in the spinal cord can be blocked by descending inhibitory pathways from the midbrain and by sensory Aβ fibers arising in peripheral tissues. These two systems constitute the neurologic basis of the gate-control hypothesis.
According to this hypothesis, pain transmission by spinothalamic neurons can be modulated, or gated, by the inhibitory activity of other types of large fibers impinging on them. The activation of spinothalamic neurons is also inhibited by peripheral Aβ sensory fibers that stimulate the release of enkephalins from spinal cord interneurons. The descending inhibitory pathways arise from periaqueductal gray (PAG) in the mid brain and they project to medullary nuclei that transmit impulses to the spinal cord. The medullary neurons include serotonergic nerves arising in the nucleus magnus raphae (NMR) and noradrenergic nerves arising in the locus ceruleu (LC). When these nerves release serotonin and norepinephrine in the spinal cord, they inhibit dorsal spinal neurons that transmit pain impulses to supraspinal sites. Nerve fibers from the PAG also activate spinal interneurons that release an endogenous opioid peptide, met-enkephalin. The enkephalins act presynaptically to decrease the release of pain transmitters from the central terminations of primary afferent neurons. They also act on postsynaptic receptors on spinothalamic tract neurons in the spinal cord to decrease the rostral transmission of the pain signal.45

2.12 Opioid Peptides and Receptors:

The presence of stereoselective receptors for morphine in brain tissue indicated the likelihood of an endogenous ligand for these receptors, and these eventually led to discovery of three major families of endogenous ligand for these receptors, and this eventually led to
the discovery of the three major families of endogenous opioid peptides.

- Enkephalins
- Endorphins
- Dynorphins

Opioid peptides are derived from larger precursor molecules encoded by separate genes; proenkephalin, proopiomelanocortin and prodynorphin respectively. Enkephalergic interneurons in the dorsal horn produce presynaptic inhibition of primary afferent neurons and post synaptic inhibition of secondary neurons and post synaptic inhibition of secondary neurons in ascending pathways. Endorphin and dynorphins are large peptides. The two types of enkephalins are small pentapeptides containing Try-Gly-Gly-Phe-Met/Leu. Hence, the two types of enkephalins are called Met-enkephalin and Leu-enkephalin.

2.13 **Met-enkephalin** and **Leu-enkephalin**.\(^{51}\)

The enkephalins are released from neurons throughout the pain axis, including those in the PAG, medulla, and spinal cord. Enkephalins activate opioid receptors in these areas and thereby block the transmission of pain impulses. The enkephalins appear to act as neuromodulators in that they exert a long-acting inhibitory effect on the release of excitatory neurotransmitters by several neurons.

The three opioid receptor types are

\(\mu\) (mu) receptors,
δ(delta) receptors,
κ(Kappa) receptors

Most of the clinically useful opioid analgesics, however, have preferential or strong selectivity of μ receptors.\(^{45}\)

2.13.1 Diagnosis:

There is no way to tell how much pain a person has. No test can measure the intensity of pain, no imaging device can show pain, and no instrument can locate pain precisely. Sometimes, as in the case of headaches, physicians find that the best aid to diagnosis is the patient's own description of the type, duration, and location of pain. These descriptions are part of what is called the pain history, taken by the physician during the preliminary examination of a patient with pain. Physicians, however, do have a number of technologies to find the cause of pain. Primarily these include:

Electro diagnostic procedures include electromyography (EMG), nerve conduction studies, and evoked potential (EP) studies, Imaging, especially Magnetic resonance imaging (MRI), provides physicians with pictures of the body's structures and tissues.

2.14 Management of Pain

The goal of pain management is to improve function, enabling individuals to work, attend school, or participate in other day-to-day activities. Patients and their physicians have a number of options for the treatment of pain; some are more effective than others. Sometimes, relaxation and the use of imagery as a distraction provide relief. These methods can be powerful and effective, according to those
who advocate their use. Whatever the treatment regime, it is important to remember that pain is treatable. The following treatments are among the most common.

**Acupuncture** dates back 2,500 years and involves the application of needles to precise points on the body. It is part of a general category of healing called traditional Chinese or Oriental medicine.

**Cognitive-behavioral therapy** involves a wide variety of coping skills and relaxation methods to help prepare for and cope with pain. It is used for postoperative pain, cancer pain, and the pain of childbirth.

**Electrical stimulation**, including transcutaneous electrical stimulation (TENS), implanted electric nerve stimulation, and deep brain or spinal cord stimulation, is the modern-day extension of age-old practices in which the nerves of muscles are subjected to a variety of stimuli, including heat or massage. Electrical stimulation, no matter what form, involves a major surgical procedure and is not for everyone, nor is it 100 percent effective. The following techniques each require specialized equipment and personnel trained in the specific procedure being used.

**Tens** uses tiny electrical pulses, delivered through the skin to nerve fibers, to cause changes in muscles, such as numbness or contractions. This in turn produces temporary pain relief. There is also evidence that TENS can activate subsets of peripheral nerve
fibers that can block pain transmission at the spinal cord level, in much the same way that shaking your hand can reduce pain.

**Peripheral nerve stimulation** uses electrodes placed surgically on a carefully selected area of the body. The patient is then able to deliver an electrical current as needed to the affected area, using an antenna and transmitter.

**Spinal cord stimulation** uses electrodes surgically inserted within the epidural space of the spinal cord. The patient is able to deliver a pulse of electricity to the spinal cord using a small box-like receiver and an antenna taped to the skin.

**Deep brain or intracerebral stimulation** is considered an extreme treatment and involves surgical stimulation of the brain, usually the thalamus. It is used for a limited number of conditions, including severe pain, central pain syndrome, cancer pain, phantom limb pain, and other neuropathic pains. **Exercise** has come to be a prescribed part of some doctors’ treatment regimens for patients with pain. Because there is a known link between many types of chronic pain and tense, weak muscles, exercise—even light to moderate exercise such as walking or swimming—can contribute to an overall sense of well-being by improving blood and oxygen flow to muscles. Just as we know that stress contributes to pain.

**Hypnosis and relaxation** are prominent ancillary clinical techniques in the treatment of acute and chronic pain. Two prominent psychological treatments Hypnosis and relaxation training, to the clinical reduction of pain, the findings from acute pain studies show
hypnosis treatment to be superior to attention or standard care control conditions, and often superior to other viable pain treatments. The findings from chronic pain studies suggest that hypnotic treatment is consistently superior to non-treatment, and often as effective but not superior to other viable treatments. There are so many types of drugs used to relieve pain, include Opioid/narcotic/morphine like analgesics, Natural opium alkaloids, Semi synthetic opiates, Synthetic opioids, Complex action opioids and opioid antagonists are not used as analgesics: Nalorphine, Levallorphan, some opioid antagonists are used as analgesic: Pentazocine, Nalbuphine, Buprenorphine, butophanol, Naloxone, Naltrexone. Second one is Non-opioid/non-narcotic/asprin like/antipyretic or anti-inflammatory analgesics in these Salicylates, pyrazolone derivatives, indole derivatives, propionic acid derivatives, anthranilic acid derivatives, aryl-acetic derivative, Oxicam derivatives, pyrrolo-pyrrole derivative, sulfonamide derivative, alkanones and third one is analgesic but poor anti-inflammatory activity those are paraaminophenol derivatives, pyrazolone derivatives, benzoazocine derivative.52

2.15 Herbal Therapies

People are looking for safe, effective alternatives for pain and herbal remedies have the advantage of fewer side effects. The following herbal remedies have been known to provide pain relief:

• Capsaicin is found naturally in cayenne pepper. (Its cream or gel form may be able to relieve some arthritic pain.)
• Bromelin reduces inflammation.
• Curcumin reduces inflammation.
• Willow bark reduces inflammation
• Pine-bark and grape-seed extracts: reduces inflammation.\textsuperscript{53}

2.16 Animal Models to Screen Analgesic Activity:

Central Analgesic Activity

\textbf{In - Vitro Methods:\textsuperscript{54}}

1. \textsuperscript{3}H-Naloxone binding assay
2. \textsuperscript{3}H-Dihydromorphine binding to \(\delta\) opiate receptors in rat brain
3. \textsuperscript{3}H- Bremazocine binding to \(\kappa\) opiate receptors in Guinea pig cerebellum
4. Inhibition of enkephalinase

\textbf{In -Vivo Methods}

1. HAFFNER’S tail clip method
2. Radiant heat method
3. Hot plate method
4. Tail immersion method
5. Electrical stimulation of the tail
6. Grid shock test
7. Formalin test in rats

Peripheral Analgesic Activity

1. Writhing tests
2. Pain in inflamed tissue (RANDALL-SELITTO-test)
3. Effect of analgesics on spinal neurons
2.17 Inflammation

Inflammation is defined as the local response of living mammalian tissues to tissue injury due to any agent. It is a body defense reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent necroosed cells and tissues.

2.17.1 Signs of Inflammation

Rubor (redness), Tumor (swelling), Calor (heat), Dolor (pain), Function laesa (loss of function)

2.17.2 Types of Inflammation

1. Acute inflammation
2. Chronic inflammation

2.17.2.1 Acute Inflammation

Acute inflammation is of short duration and represents the early body reaction. The changes in acute inflammation can be conveniently described under the following two headings:

I. Vascular events
II. Cellular events

a. Vascular events

Alteration in the microvasculature (arterioles, capillaries and venules) is the earliest response to tissue injury. These alterations include: haemodynamic changes and changes in vascular permeability.

b. Haemodynamic changes

The earliest features of inflammatory response result from changes in the vascular flow and caliber of small blood vessels in
injured tissue. The features of haemodynamic changes in inflammation are best demonstrated by the Lewis experiment. Lewis induced the changes in the skin of inner aspect of forearm by firm stroking with a blunt point. The reaction so elicited is known as triple response or red line response consisting of the following:

i) Red line appears within a few seconds following stroking and results from local vasodilatation of capillaries and venules.

ii) Flare is the bright reddish appearance or flush surrounding the redline and results from vasodilatation of the adjacent arterioles.

iii) Wheal is the swelling or edema of the surrounding skin occurring due to transudation of fluid into the extravascular space. These features, thus, elicit the classical signs of inflammation, redness, heat, swelling and pain.

c. Vascular permeability

In and around the inflamed tissue there is accumulation of edema fluid in the interstitial compartment which comes from blood plasma by its escape through the endothelial wall of peripheral vascular bed. In the initial stage, the escape of fluid is due to vasodilatation and consequent elevation in hydrostatic pressure. This is transudate in nature. But subsequently, the characteristic inflammatory edema, exudates, appears by increased vascular permeability of microcirculation.

a. Cellular events

The cellular phase of inflammation consists of 2 processes:

a) Exudation of leucocytes
b) Phagocytosis

b. Exudation of leucocytes

The escape of leucocytes from the lumen of microvasculature to the interstitial tissue is the most important feature of inflammatory response. In acute inflammation, polymorphonuclear neutrophils (PMNs) comprise the first line of body defense, followed later by monocytes and macrophages.

c. Phagocytosis

Phagocytosis is defined as the process of engulfment of solid particulate material by the cells (cell-eating). The cells performing this function are called phagocytes. There are 2 main types of phagocytic cells:

i) Polymorphonuclear neutrophils (PMNs) which appear early in acute inflammatory response also called as microphages.

ii) Circulating monocytes and fixed tissue mononuclear phagocytes called as macrophages.

2.17.3 Chemical mediators of inflammation

I. Cell derived mediators

1. Vasoactive amines (Histamine, 5-HT)

2. Arachidonic acid metabolites

   i. Metabolites via cyclo-oxygenase pathway
      (prostaglandins, thromboxane A2, prostacyclin)

   ii. Metabolites via lipo-oxygenase pathway (5-HETE, leukotrienes)

3. Lysosomal components
4. Platelet activating factor

5. Cytokines (IL-1, TNF-α, TNF-β, IF-γ, chemokines)

6. Nitric oxide and oxygen metabolites

II. Plasma derived mediators (plasma proteases)

These are products of:

1. The kinin system
2. The clotting system
3. The fibrinolytic system
4. The complement system

2.17.4 The inflammatory cells

The cells participating in acute and chronic inflammation are circulating leucocytes, plasma cells and tissue macrophages.

a. Polymorphonuclear Neutrophils (PMN)

Commonly called as neutrophils or polymorphs, these cells along with basophils are known as granulocytes due to the presence of granules in the cytoplasm. These granules contain many proteases, myeloperoxidase, lysozyme, esterase, aryl sulfatase, acid and alkaline phosphatase and cationic proteins. The diameter of neutrophils ranges from 10-15 µm and are actively motile. These cells comprise 40-75% of circulating leucocytes and their number is increased in blood and tissues in acute bacterial infections. These cells arise in the bone marrow from stem cells. The functions of neutrophils in inflammation are as follows:

i) Initial phagocytosis of micro-organism as they form the first line of body defense in bacterial infection.
ii) Engulfment of antigen-antibody complexes and non-microbial material.

iii) Harmful effect of neutrophils is destruction of the basement membranes of glomeruli and small blood vessels.

b. **Eosinophils**

These are larger than neutrophils but are fewer in number, comprising 1-6% of total blood leucocytes. Eosinophils share many structural and functional similarities with neutrophils like their production in the bone marrow, locomotion, phagocytosis, lobed nucleus and presence of granules in the cytoplasm containing a variety of enzymes of which major basic protein and eosinophil cationic protein are the most important which have bactericidal and toxic action against helminthic parasites.

c. **Basophils**

The basophils comprise about 1% of circulating leucocytes and are morphologically and pharmacologically similar to mast cells of tissue. These cells contain coarse basophilic granules in the cytoplasm and a polymorphonuclear nucleus. The roles of these cells in inflammation are:

i) In immediate and delayed type hypersensitivity reactions

ii) Release of histamine by IgE-sensitised basophils

d. **Lymphocytes**

Next to neutrophils, these cells are most numerous of the circulating leucocytes (20-45%). Apart from blood, lymphocytes are present in large numbers in spleen, thymus, lymph nodes and
mucosa-associated lymphoid tissue (MALT). They have scanty cytoplasm and consist almost entirely of nucleus. Besides their role in antibody formation and in cell-mediated immunity these cells participate in the following types of inflammatory responses:

i) In tissues, they are dominant cells in chronic inflammation and late stage of acute inflammation.

ii) In blood, their number is increased (lymphocytosis) in chronic infections like tuberculosis.

e. Plasma cells

These cells are larger than lymphocytes with more abundant cytoplasm and an eccentric nucleus which has cart-wheel pattern of chromatin. Plasma cells are not seen in peripheral blood. They develop from lymphocytes and are rich in RNA and γ-globulin in their cytoplasm.

f. Mononuclear-phagocyte system (Reticulo endothelial system)

This cell system includes cells derived from 2 sources with common morphology, function and origin. These are as under:

i) Blood monocytes: Thes comprises 4-8% of circulating leucocytes.

ii) Tissue macrophages

Role of macrophages in inflammation

i) Phagocytosis (cell eating) and Pinocytosis (cell drinking)

ii) Macrophages on activation by lymphokines released by T lymphocytes or by non-immunologic stimuli elaborate a variety of biologically active substances like proteases, plasminogen activators,
products of complement, some coagulation factor, chemotactic agent etc.

**g. Giant cells**

When the macrophages fail to deal with particles to be removed, they fuse together and form multinucleated giant cells. Various types of giant cells seen in inflammation and in certain tumors. These are foreign body giant cells, Langhans’ giant cells, Touton giant cells, Tumor giant cells etc.

**2.17.4 Fate of acute inflammation**

The acute inflammatory process can culminate in one of the following 4 outcomes:

1) Resolution

2) Healing by scaring

3) Progression to suppuration

4) Progression to chronic inflammation

**2.18 Chronic Inflammation**

Chronic inflammation is defined as prolonged process in which tissue destruction and inflammation occur at the same time.

**2.18.1 Causes of chronic inflammation**

a. **Chronic inflammation following acute inflammation**: when the tissue destruction is extensive or the bacteria survive and persist in small numbers at the site of acute inflammation e.g.: in osteomyelitis, pneumonia terminating in lung abscess.

b. **Recurrent attacks of acute inflammation**: when repeated bouts of acute inflammation culminate in chronicity of the process e.g. in
recurrent urinary tract infection leading to chronic pyelonephritis, repeated acute infection of gall bladder leading to chronic inflammation.

c. **Chronic inflammation starting de novo:** when the infection with organism of low pathogenicity is chronic from the beginning e.g. infection with *mycobacterium tuberculosis*.

### 2.18.2 General features of chronic inflammation

Though there may be differences in chronic inflammatory response depending upon the tissue involved and causative organisms, there are some basic similarities amongst various types of chronic inflammation. These general features characterize any chronic inflammation.

a. **Mononuclear cell infiltration:** Chronic inflammatory lesions are infiltrated by mononuclear inflammatory cells like phagocytes and lymphoid cells. Phagocytes are represented by circulating monocytes, tissue macrophages, epithelioid cells and sometimes, multinucleated giant cells. The macrophages comprise the most important cells in chronic inflammation.

b. **Tissue destruction or necrosis:** Tissue destruction and necrosis are common in many chronic inflammatory lesions and are brought about by activated macrophages by release of a variety of biologically active substances.

c. **Proliferative changes:** As a result of necrosis, proliferation of small blood vessels and fibroblasts is stimulated resulting in formation of
inflammatory granulation tissue. Eventually, healing by fibrosis and collagen laying takes place.

2.18.3 Types of chronic inflammation

i. Nonspecific: when the irritant substances produces a non-specific chronic inflammatory reaction with formation of granulation tissue and healing by fibrosis e.g. chronic osteomyelitis, chronic ulcer.

ii. Specific: when the injurious agent causes a characteristic histologic tissue response e.g. tuberculosis, leprosy, syphilis. However, for a more descriptive classification, histological features are used for classifying chronic inflammation into 2 corresponding types;

   a. Chronic nonspecific inflammation: It is characterized by nonspecific inflammatory cell infiltration e.g. chronic osteomyelitis, lung abscess.

   b. Chronic granulomatous inflammation: it is characterized by formation of granulomas e.g. tuberculosis, leprosy, syphilis etc.55

2.18.4 Mechanism of action of non-steroidal anti-inflammatory drugs:

Several hypotheses have been put forward to explain the action of Asprin like drugs:

The different hypotheses include the following:

1. Interference with oxidative phosphorylation

2. Displacement of an endogenous anti-inflammatory peptide from plasma protein

3. Interference with the migration of leukocytes
In 1971 Vane and coworkers made the landmark observation that Aspirin and some NSAIDS blocked PG generation. Prostaglandins, prostacyclins (PGI2) and thromboxone A2 (TXA2) are produced from arachidonic acid by the enzyme cyclooxygenase which exists in a constitute (COX-1) and an inducible (COX-2) isoforms. COX-1 serves physiological functions, while COX-2 normally present in minute quantities, is induced by cytokinines and other signal molecules at the site of inflammation leads to generation of PGs locally which mediate many of the inflammatory changes. Most NSAIDs inhibit COX-1 and COX-2 non-selectively, but now some (celecoxib) selective COX-2 inhibitors have been produced.

The inhibition of prostaglandin synthesis is now considered to be the main mechanism of action of NSAIDS. Interestingly, most of the stimuli known to induce COX-2 are those associated with inflammation, for example, bacterial lipopolysacharide and cytokines IL-1, IL-2 and tumor necrosis Factor (TNF-α). The physiological roles of COX-1 have been deducted from the deleterious side effects of NSAIDS, which while inhibiting prostaglandin biosynthesis at inflammatory sites also inhibit constitutive biosynthesis. Thus, COX-1 provides prostaglandins in the intestine to maintain the integrity of the mucosal epithelium and its inhibition leads gastric damage,
hemorrhage and ulceration. It is suggested that the cytoprotective role of prostaglandins (e.g. Prostacyclin PGI2) in the stomach is largely due to their vasodilating properties, enhancing mucosal blood flow. The discovery and characterization of COX-2 have clarified some long-standing puzzles. One problem in therapeutics appears to have been solved – suppression of inflammation without the untoward effects of NSAIDS, namely, gastrointestinal ulceration renal damage and platelet dysfunction were regarded as inevitable consequences of the inhibition of COX activity required to prevent synthesis of prostaglandins in inflammatory conditions such as rheumatoid arthritis. However with COX-2 clearly associated with inflammation, but not with the physiological synthesis of prostaglandins, selective inhibitors of COX-2 have offered the possibility of inhibition of inflammatory prostaglandins without affecting prostaglandins generated by COX-1, in the stomach, kidney or platelet.56

2.19 Animal Models to Screen Anti-Inflammatory Activity

In vitro methods54

1. 3H-Bradykinin receptor binding
2. Substance P and the tachykinin family
3. 3H- substance P receptor binding
4. Neurokinin receptor binding 5. Assay of polymorph nuclear leukocyte chemotaxis in vitro
6. Constitute and inducible cellular arachidonic acid metabolism in vitro
7. COX-1 and COX-2 inhibition
In vivo methods

1. Methods for testing acute and subacute inflammation
2. Ultraviolet erythema in guinea pigs
3. Vascular permeability
4. Rat paw oedema
5. Pleurisy test
6. Granuloma pouch technique
7. Cotton wool granuloma
8. Measurement of gastric mucosal damage by intragastric inulin

Table No 2.2: Medicinal Plants Possessing Anti-inflammatory and Analgesic activity

<table>
<thead>
<tr>
<th>S.No</th>
<th>Plant</th>
<th>Part used</th>
<th>Extract</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Bibersteinia mutilifada</td>
<td>Root</td>
<td>Ethanol</td>
<td>Hassan Farsam et al.,(57)</td>
</tr>
<tr>
<td>2.</td>
<td>Chasmanthera dependens</td>
<td>Leaves</td>
<td>Methanol</td>
<td>Olugbenga Morebise et al.,(58)</td>
</tr>
<tr>
<td>3.</td>
<td>Cissus quadrangularis</td>
<td>Aerial parts</td>
<td>Methanol</td>
<td>Ampai panthong et al.,(59)</td>
</tr>
<tr>
<td>4.</td>
<td>Curatella Americana</td>
<td>Bark</td>
<td>Hydroalcoholic</td>
<td>M.S.Alexandere-Moreiraet et al.,(60)</td>
</tr>
<tr>
<td>5.</td>
<td>Cussonia paniculata</td>
<td>Stem bark</td>
<td>Aqueous</td>
<td>Adeolu A.Adedapo et al.,(61)</td>
</tr>
<tr>
<td>7.</td>
<td>Diospyros variegate</td>
<td>Stem</td>
<td>Hexane</td>
<td>S.Trongsakul et al.,(63)</td>
</tr>
<tr>
<td>8.</td>
<td>Erythrophleum suaveolens</td>
<td>Stem bark</td>
<td>Methanol</td>
<td>A.B.Dongmo et al.,(64)</td>
</tr>
<tr>
<td>9.</td>
<td>Feronia limonia</td>
<td>Fruit pulp</td>
<td>Methanol</td>
<td>S.Mansoor Ahamed et al.,(65)</td>
</tr>
<tr>
<td>10.</td>
<td>Hypericum empetrifolium</td>
<td>Aerial parts</td>
<td>Methanol</td>
<td>Ada Trovato et al.,(66)</td>
</tr>
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<td>11.</td>
<td>Glaucium grandiflorum</td>
<td>Aerial parts</td>
<td>Methanol</td>
<td>K.Morteza-Semnani et al.,(67)</td>
</tr>
<tr>
<td>12.</td>
<td>Glycine tomentella</td>
<td>Root</td>
<td>Aqueous</td>
<td>Tsung-Chun Lu et al.,(68)</td>
</tr>
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<td></td>
<td>Plant Name</td>
<td>Part(s)</td>
<td>Extraction Method</td>
<td>Authors</td>
</tr>
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<td>-------------------</td>
<td>----------------------------------------------</td>
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<tr>
<td>13</td>
<td>Heracleum persicum</td>
<td>Fruits</td>
<td>Hydroalcohol</td>
<td>Valiollag Hajhashemi et al.,(69)</td>
</tr>
<tr>
<td>14</td>
<td>Lactuca sativa</td>
<td>Seeds</td>
<td>Methanol &amp; pet.ether</td>
<td>Mohammad Sayyah et al.,(70)</td>
</tr>
<tr>
<td>15</td>
<td>Lavandula angustifolia</td>
<td>Leaves</td>
<td>Hydroalcohol &amp; Essential oil</td>
<td>Valiollag Hajhashemi et al.,(71)</td>
</tr>
<tr>
<td>16</td>
<td>Ligularis fischeri</td>
<td>Leaves</td>
<td>Ethanol</td>
<td>Kyung-Hee Lee et al.,(72)</td>
</tr>
<tr>
<td>17</td>
<td>Mallotus spodocarpus</td>
<td>Root</td>
<td>Chloroform</td>
<td>S.Intahphuak et al.,(73)</td>
</tr>
<tr>
<td>18</td>
<td>Nelsonia canescens</td>
<td>Leaves</td>
<td>Ethanol</td>
<td>Victor B. Owoyele et al.,(74)</td>
</tr>
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<td>19</td>
<td>Potomorphe umbellata</td>
<td>Aerial parts</td>
<td>Hydroalcohol</td>
<td>Fabio F.Perazzo et al.,(75)</td>
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<tr>
<td>20</td>
<td>Persea Americana</td>
<td>Leaves</td>
<td>Aqueous</td>
<td>O.O.Adeyemi et al.,(76)</td>
</tr>
<tr>
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<td>Peperomia pellucid</td>
<td>Aerial parts</td>
<td>Aqueous</td>
<td>Dmitrieva et al.,(77)</td>
</tr>
<tr>
<td>22</td>
<td>Rosa multiflora</td>
<td>Fruits</td>
<td>Ethanol</td>
<td>Guang-qin Zhang et al.,(78)</td>
</tr>
<tr>
<td>23</td>
<td>Sida cordifolia</td>
<td>Leaves</td>
<td>Aqueous</td>
<td>E.M. Franzotii et al.,(79)</td>
</tr>
<tr>
<td>24</td>
<td>Tithonia Diversifolia</td>
<td>Leaves</td>
<td>Methanol</td>
<td>Victor B. Owoyele et al.,(80)</td>
</tr>
<tr>
<td>25</td>
<td>Vitex negundo</td>
<td>Leaves</td>
<td>Aqueous</td>
<td>M.G.Dharmasiri et al.,(81)</td>
</tr>
</tbody>
</table>

**2.20 Literature Review of *Citrus maxima* (J.Burm.) Merr. [82-85]**

**2.20.1 PLANT PROFILE:**

**Botanical Name:** *Citrus maxima* (J.Burm.) Merr.

**Synonym:** *Citrus grandis* (L).

*Citrus decumana* Watt.

**Family:** Rutaceae

**Common Names:** Pomelo, Chinese grapefruit, Pummelo, Pommelo, Jabong, Shaddock.

**Vernacular names:**
- Hindi: Sadaphal, Batawi nimbu, Cakotaraa
- Manipuri: Nobab
- Tamil: Pambalimasu
- Malayalam: Pamparamasan
Telugu: Pampara
Bengali: Chakotra
Konkani: Toranji
Sanskrit: Madhukarkati
Kannada: Chakkota

**Distribution:** India, China, Japan, Indonesia, United state of America, Philippine, Thailand.

![Fig. 2.8: Plant and Leaf of *Citrus maxima* (J.Burm.) Merr](image1)

![Fig. 2.9: Fruit and fruit peel of *Citrus maxima* (J.Burm.) Merr.](image2)
2.20.2 Plant Description:

The *Citrus maxima* tree, which is the most cold-intolerant citrus species, has a rounded crown and grows 5 to 15 m (15 to 50 ft) tall. The tree has large evergreen oblong to elliptic leaves, 10.5 to 20 cm (4 to 8 in) long, with winged petioles (leaf stems). The flowers and fruits are borne singly, in contrast to grape fruits (*C. X paradisi*), in which they grown in clusters of 2 to 20. The fruits, which vary from round to pear-shaped and ripen to yellow, orange, or red, are large--30 cm or more in diameter, and weighing up to 9 kg (20 lbs). The flesh of the fruit, which may be greenish yellow, yellow, pink, or red, is often juicy, and divided into 11 to 18 segments. The flavor is sweet to somewhat acidic.

2.20.3 Traditional Uses:

The leaves, flowers, and rind are given for their sedative effect in cases of epilepsy, chorea and convulsive coughing. The hot leaf decoction is applied on swellings and ulcers. The fruit juice is taken as a febrifuge. The seeds are employed against coughs, dyspepsia and
lumbago. The fruit include treatment of coughs, fevers, cardiotonic, cancer and gastrointestinal disorders. The plant used as anti diarrhea.

2.20.4 Chemical constituents: 86-92

*Citrus* fruits are very rich source of flavonoids like hespiridin, naringin. The peels of the fruits are richer sources of hydroxy cinnamic acid than juice and pectin. Flavonoids hespiridin and naringin are found to be present in the peel and inner part of the fruit of *Citrus limetta*. The essential oils α-pinene, β-pinene, sabinene, β-myrcene, p-cymene, limonene, γ-terpinene, neryl acetate, β-bisabolene, α-bergamotene.

The major flavanones of *Citrus maxima* are neohesperidin and naringin, which are high in the seed than in unripe fruits. Hesperidin, naringin, caffeic, p-coumaric, ferulic and vanillic acid are present in the fruit juice. A C-C linked bisacridone alkaloid buntanbismine, was isolated from the stem bark of *C. grandis*. *Citrus maxima* essential oil is composed of α-pinene, sabinine, β-pinine, methyl heptenone, β-myrcene, hexanal, sabinine, DL-limonine, t-ocimine, linalool, 1-hexene, 4-methyl; 1-hexene,3,3-dimethyl; geranyl formate, Z-citral, geranyl formate, E-citral, geranyl acetate, β-farnesene. Fruits are also the richest source of ascorbic acid which varies from 50-100 mg per 100 mL or g of edible portion.

2.21 Literature Review of *Citrus aurantium* Linn. 93-95

2.21.1 PLANT PROFILE:

**Botanical Name:** *Citrus aurantium* Linn.

**Family:** Rutaceae

**Common Names:** Bigarade Orange, Bitter Orange, Seville orange (sweet), Portugal Orange, Citrus Dulcis.

**Vernacular names:** Sanscrit: Nagaruka, Naranga  
Tamil : Narandam, Nagarukam  
Hindi : Narangi, Santara  
Telugu: Vadalapudi, Kichli  
Kannada: Elikai

**Distribution:** India, China. Cultivated in Spain, Madeira

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*Fig. 2.11: Plant and Leaf of Citrus aurantium Linn.*
Fig. 2.12: Fruit and fruit peel of *Citrus aurantium* Linn.

Fig. 2.13: Bark of *Citrus aurantium* Linn.

**2.21.2 Plant Description:**

The tree ranges in height from less than 10 ft (3 m) to 30 ft (9 m), is more erect and has a more compact crown than the sweet orange; has smooth, brown bark, green twigs, angular when young, and flexible, not very sharp, thorns from 1 in to 3 1/8 in (2.5-8 cm) long. The evergreen leaves (technically single leaflets of compound leaves), are aromatic, alternate, on broad-winged petioles much longer than those of the sweet orange; usually ovate with a short point at the apex; 2 1/2 to 5 1/2 in (6.5-13.75 cm) long, 1 1/2 to 4 in (3.75-10 cm)
wide; minutely toothed; dark-green above, pale beneath, and dotted with tiny oil glands. The highly fragrant flowers, borne singly or in small clusters in the leaf axils, are about 1 1/2 in (3.75 cm) wide, with 5 white, slender, strap like, recurved, widely-separated petals surrounding a tuft of up to 24 yellow stamens. From 5 to 12% of the flowers are male.

The fruit is round, oblate or oblong-oval, 2 3/4 to 3 1/8 in (7-8 cm) wide, rough-surfaced, with a fairly thick, aromatic, bitter peel becoming bright reddish-orange on maturity and having minute, sunken oil glands. There are 10 to 12 segments with bitter walls containing strongly acid pulp and from a few to numerous seeds. The center becomes hollow when the fruit is full-grown.

**2.21.3 Traditional Uses: 96**

China - Abdomen, Ache, Antidote, Anodyne, Antiseptic, Anti-inflammatory, Bactericide, Bubo, Cancer, Cancer (Breast), Cancer (Stomach), Carminative, Chest, Congestion, Deobstruent, Diarrhea, Dysmenorrhea, Dyspepsia, Dyspnea, Emmenagogue, Freckle, Fungicide, Gas, Prolapse, Nausea, Marasmus, Panacea, Pectoral, Pimple, Rectocele, Refrigerant, Rib, Sedative, Sore, Spasm, Splenitis, Stomach, Stomachic, Thirst, Urinogenital, Uterus, Vermifuge, Wine-Nose.

Curacao - Gall-Bladder, Hypertension, Nerve, Shampoo, Tea, Tranquilizer.

Elsewhere - Ache (Stomach), Antifertility, Carminative, Carminative, Chest, Expectorant, Emmenagogue, Gall-Bladder, Heart, Hemostat,
Medicine, Nerve, Spasm, Stimulant, Stomach, Stomachic, Styptic, Sudorific, Tonic.

Haiti - Antiseptic, Fever, Laxative, Purgative.

India - Ache (Stomach), Hypertension, Liver, Megalospleny, Menorrhagia

Mexico - Ache, Antiseptic, Apertief, Nerve Tonic, Tranquilizer, Tea

Trinidad - Depurgative, Dyspepsia, Expectorant, Flatulence, Mouthwash, Oliguria, Purgative, Sedative, Sore, Thrush.

Turkey - Antiseptic, Aperitif, Narcotic, Nervine, Sedative, Scurvy, Stomachic, Tonic

US - Cancer, Fatality.

2.21.4 Chemical constituents: 

Bitter orange has a complex chemical makeup, though it is perhaps most known for the volatile oil in the peel. The familiar oily residue that appears after peeling citrus fruit, including bitter orange, is this volatile oil. It gives bitter orange its strong odor and flavor, and accounts for many of its medicinal effects. Besides the volatile oil, the peel contains flavones, the alkaloids synephrine, octopamine, and N-methyltyramine, and carotenoids.

Main content: synephrine, $C_9H_{13}NO_2$, 0.24 to 1.45% (g/g); N-methyltyramine, $C_9H_{13}NO$, 0.19 to 0.83% (g/g). Other Phytochemicals: neohesperidin; nobiletin; 5-odesmethyl nobiletin; quinoline; narcotin; noradrenline; tryptamine; tyramine; naringin; rhoifolin; lonicerin. C. sinensis Osbeck (Sweet orange) a variety of citrus also contains hesperitin; naringenin; isosakuranetin; carotene; riboflavine;
tengeretin; 3, 5, 6, 7, 8, 3’4’-methoxyflavone. Fruit peel contains volatile oil composed of d-limonene; d-linalool, N-acetyloctopamine; gamma-aminobutyric acid.

Bitter orange peel contains a volatile oil with limonene (about 90%), flavonoids, coumarins, triterpenes, vitamin C (ascorbic acid) varied from 11-19 mg per fresh weight of the edible portion, carotene, and pectin. The flavonoids have several useful properties, being anti-inflammatory, antibacterial, and antifungal. The composition of the volatile oils in the leaves, flowers, and peel varies significantly. Linalyl acetate (50%) is the main constituent in oil from the leaves (petit grain), and linalool (35%) in oil from the flowers (neroli). The unripe fruit of the bitter orange contains cirantin, which reputedly is a contraceptive. Include: (+)-auraptenal, 4-terpineol, 5-hydroxyauranetin, acetaldehyde, acetic-acid, alphanhumulene, alpha-ionone, alpha-phellandrene, alphapinene, alpha-terpineol, alpha-terpinyl-acetate, alphaylangene, ascorbic-acid, aurantiamicene, aurapten, benzoic acid, beta-copaene, beta-elemene, betaocimene, beta-pinene, butanol, cadinene, camphene, caprinaldehyde, carvone, caryophyllene, cinnamic acid, cis-oicime, citral, citronellal, citronellic acid, citronellol, cryptoxanthin, d-citronellic acid, d-limonene, d-linalool, dnerolidol, decanal, decylaldehyde, decylpelargonate, delta-3-carene, delta-cadinene, dipentene, dl-linalool, dlterpineol, dodecanal, dodecen-2-al- (1), duodeclyaldehyde, ethanol, farnesol, formic acid, furfurol, gamma-elemene, gammaterpinene, geranic acid, geraniol, geranyl acetate, geranyl oxide,
hesperidin, hexanol, indole, isolimonic acid, isoscutellarein, isosinensetin, isotetramethylether, l-linalool, linalylacetate, l-stachydrine, lauric aldehyde, limonene, limonin, linalool, linalyl acetate, malic acid, mannose, methanol, myrcene, naringenin, naringin, neral, nerol, nerolidol, neryl acetate, nobiletin, nomilin, nonanol, nonylaldehyde, nootkatone, octanol, octyl acetate, p-cymene, p-cymol, palmitic acid, pectin, pelargonic acid, pentanol, phellandrene, phenol, phenylacetic acid, pyrrol, pyrrole, rhoifolin, sabinene, sinensetin, stachydrine, tangeretin, tannic acid, terpenyl acetate, terpinen-4-ol, terpinolene, tetra-o-methyl-scutellarein, thymol, transhexen-2-al-1, trans-ocimene, umbelliferone, undecanal, valencene, and violaxanthin.