CHAPTER I

Introduction
Chapter 1

INTRODUCTION

The doctrine of evolution tends to magnify the competitive aspects of nature and to minimize the cooperative aspects. "Nature red in tooth and claw" is indeed the theme of many evolutionary books. However, the living world around us has many examples of cooperative or symbiotic behavior at many different levels. We need look no further than our own bodies for some of the most marvelous instances of cooperative activity in all of creation. The processes of breathing, digestion, muscular contraction and coordination, nutrient transport, and their direction by the neural and endocrine systems, have shown an amazing sophistication of cooperation through molecular biologic research.

The last two decades have witnessed the growth of new inter-disciplinary fields termed ethnobotany, phytochemistry, ethnoparmacognosy or ethnopharmacology. Higher plants, as sources of medicinal compounds continue to play a dominant role in maintenance of human health since antiquities. Over 50% of all modern clinical drugs are of natural product origin(1) and natural products play an important role in drug development programs of the pharmaceutical industry(2,3). In developing countries, especially in rural contexts people usually turn to traditional healers when in diseased conditions and plants of ethnobotanical origin are often presented for use. Throughout the ages humans have relied on nature for their basic needs for the production of foodstuffs, shelters, clothing, means of transportation, fertilizers, flavors and fragrances, and not least, medicines. Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years(4).

Although herbal medicinal products represent a fast growth area of health-care products for both humans and companion animals(5,6), their use in humans appears to predate recorded history(7). Most herbal medicines of current
interest have come from ancient civilizations of Africa, the Asian subcontinent, and North, Central and South America(8). Although use of medicinal herbs appears to be much more prevalent among humans than animals, understanding the prominence in human use is inextricably bound to perspectives gleaned from studies of self-medication in animals and differences and similarities between humans and animals in the types of herbal medicine practiced.

Investigations into the chemical and biological activities of plants during the past two centuries have yielded compounds for the development of modern synthetic organic chemistry and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents(9). Presently, many scientists and organizations are in search of traditional remedies as alternate medicine(10,11). Thus many plants that are used in traditional practice are sold in a rounded urban settlement to meet the need of a public desire for herbals, which has resulted in the industrialization, and large-scale production of a great number of products of botanical origin widely consumed.

The Reasons for Herbal Consumption:

1. Most of herbal users do not inform their allopathic doctors about CAM (complimentary and alternative medicine) intake.

2. Herbals are generally considered a traditional health aid which does not require stringent preclinical and clinical assessments by appropriate regulatory agencies.

3. Currently no proper surveillance procedure exists for quality control and for monitoring adverse effects of herbs or herb–drug combinations.

4. A general misconception persists among most CAM users that herbal medicines are safe and free from side effects and drug interactions, because these products are of natural origin(12).
Cancer:

Cancer claims over six million lives each year. On a world-wide basis, cancer represents the single largest cause of death in both men and women. Bearing in mind the level of morbidity that is often affiliated with this disease, comprehension of such a high incidence is horrifying. Obviously, the treatment of cancer and most other systemic diseases involves administration of drugs.

Cancer is a generic term for malignant neoplasm, a great group of diseases occurring in all humans and animal populations and arising in all tissues composed of potentially dividing cells. The basic characteristics of cancer is the transmissible abnormality of cells that is manifested by reduced control over growth and function leading to serious adverse effects on the host through invasive growth and metastases. The word cancer comes from the Latin for crab, probably because of the way a cancer adheres to any part that it seizes upon in an obstinate manner like the crab. The actual medical term for cancer is neoplasia, which, from the Greek, means new formation. Cancers are new growths of the cells in our bodies.

Cancer cells differ from normal cells in four fundamental ways:

- Uncontrolled proliferation
- Loss of contact inhibition to slow growth
- Lack of adhesion requirement for growth
- Inability to differentiate completely.

Causes of Cancer:

Cancer develops over time when certain normal genes start mutating. Such cells multiply rapidly and become malignant. These gene mutations occur due to a complex mix of factors related to lifestyle, heredity and environment.
A) **Radiation:** High levels of radiation like those from radiation therapies and x-rays (repeated exposure) can damage normal cells and increase the risk of developing leukemia, as well as cancers of the breast, thyroid, lung, stomach and other organs.

B) **Ultraviolet (UV) Radiation:** UV radiation from the sun is directly linked to melanoma and other forms of skin cancer. These harmful rays of the sun cause premature aging and damage the skin.

C) **Viruses:** Some viruses, including hepatitis B and C, human papilloma viruses (HPV), and the Epstein Barr virus, which causes infectious mononucleosis, have been associated with increased cancer risk. Immune system diseases, such as AIDS, can make one more susceptible to some cancers.

D) **Chemicals:** Long-term exposure to chemicals such as pesticides, uranium, nickel, asbestos, radon and benzene can increase the risk of cancer. Such carcinogens may act alone or in combination with another carcinogen, such as cigarette smoke, to increase the risk of cancer and other lung diseases.

E) **Tobacco:** Cigarette smoking and regular exposure to tobacco smoke greatly increase lung cancer. Cigarette smokers are more likely to develop several other types of cancer like those of the mouth, larynx, esophagus, pancreas, bladder, kidney and cervix. Smoking may also increase the likelihood of developing cancers of the stomach, liver, prostate, colon and rectum. The use of other tobacco products, such as chewing tobacco, is linked to cancers of the mouth, tongue and throat.

F) **Alcohol:** Heavy drinkers face an increased risk of cancers of the mouth, throat, esophagus, larynx and liver. Some studies suggest that even moderate drinking may slightly increase the risk of breast cancer.

G) **Diet:** High-fat, high cholesterol diets are proven risk factors for several types of cancer such as those of the colon, uterus and prostate. Obesity may be linked
to breast cancer among older women as well as to cancers of the prostate, pancreas, uterus, colon and ovary.

**H) Others:** Twenty percent of cancers are hereditary. This means that the abnormal gene responsible for causing cancer is passed from parent to child, posing a greater risk for that type of cancer in all descendants of the family. However, just because someone has a cancer-causing gene doesn't mean they will automatically get cancer. If hereditary cancer is suspected, family members should consider genetic counseling and testing to determine their risk. If diagnosed in the early stages, such cancers are most responsive to treatment.

Signs of hereditary cancer include:

- **Genetics:** A theory exists with some scientific support, that certain smokers have a higher risk of smoking-induced lung cancer than others because of their genetic make-up.

- **Family History:** Many cancers are associated with having a family history of that cancer. Breast, ovarian, prostate and colon are some of these cancers.

- **Several Relatives with Cancer:** Approximately up to 15% of all cancers have a familial basis. That means that cancer tends to occur among members of a family. Much of the time, different types of cancer occur apparently by chance, or in association with common family habits such as cigarette smoking more likely to develop breast cancer than a woman whose close female relatives have not had breast cancer.

**Nomenclature and Classification:**

The following closely related terms might be used to designate abnormal growths:
Neoplasia and neoplasm are the scientific designations for cancerous diseases. This group contains a large number of different diseases. Neoplasms can be benign or malignant.

Cancer is a widely used word that is usually understood as synonymous with malignant neoplasm. It is occasionally used instead of carcinoma, a sub-group of malignant neoplasms. Because of its overwhelming popularity relative to 'neoplasia', it is used frequently instead of 'neoplasia', even by scientists and physicians, especially when discussing neoplastic diseases as a group.

Tumor in medical language simply means swelling or lump, neoplastic, inflammatory or other. In common language, however, it is synonymous with 'neoplasm', either benign or malignant. This is inaccurate since some neoplasms usually do not form tumors, for example leukemia or carcinoma in situ.

Cancers are classified by the type of cell that resembles the tumor and, therefore, the tissue presumed to be the origin of the tumor. The following general categories are usually accepted:

- Carcinoma: malignant tumors derived from epithelial cells. This group represents the most common cancers, including the common forms of breast, prostate, lung and colon cancer.
- Lymphoma and Leukemia: malignant tumors derived from blood and bone marrow cells
- Sarcoma: malignant tumors derived from connective tissue, or mesenchymal cells
- Mesothelioma: tumors derived from the mesothelial cells lining the peritoneum and the pleura.
- Glioma: tumors derived from glia, the most common type of brain cell
- **Germinoma**: tumors derived from germ cells, normally found in the testicle and ovary

- **Choriocarcinoma**: malignant tumors derived from the placenta.

**Treatment of Cancer**:

Cancer can be treated by surgery, chemotherapy, radiation therapy, immunotherapy, monoclonal antibody therapy or other methods. The choice of therapy depends upon the location and grade of the tumor and the stage of the disease, as well as the general state of the patient (performance status). A number of experimental cancer treatments are also under development.

Complete removal of the cancer without damage to the rest of the body is the goal of treatment. Sometimes this can be accomplished by surgery, but the propensity of cancers to invade adjacent tissue or to spread to distant sites by microscopic metastasis often limits its effectiveness. The effectiveness of chemotherapy is often limited by toxicity to other tissues in the body. Radiation can also cause damage to normal tissue.

Because "cancer" refers to a class of diseases, it is unlikely that there will ever be a single "cure for cancer" any more than there will be a single treatment for all infectious diseases.

**Cancer and Herbals**:

Plants, including herbs and their derivatives, have been used to combat a variety of ailments including cancer. Approximately five decades of systemic drug discovery and development have established a respectable armamentarium of useful chemotherapeutic agents, as well as a number of important successes in the treatment and management of human cancer(14). Nevertheless, the need for more effective antineoplastic agents remains. The most common tumors of the adults are resistant to available antineoplastic drugs(15,16) and the majority of these agents have only limited anti-solid tumor activity(16,17).

These considerations led to critical cost-versus-benefit assessments of traditional drug discovery methodologies and to many attempts to improve their
efficiency. The currently dominating strategy involves the use of high throughput chemistry and high-throughput screening(18), which allows for the rapid production and evaluation of large numbers of candidate compounds.

These limitations led to the reappraisal of another major source of chemical diversity that has consistently proven its value for the development of novel drugs: natural products(4,18,19). This switch in acquisition policy stemmed from the realization that nature, even when compared with the powerful combinatorial chemistry techniques, provides candidate compounds, which have more "drug-like" properties (i.e., in terms of absorption and metabolism) as well as a greater chemical diversity (i.e., to allow for structure-activity studies)(20). Once acquired, these substances can be entered into high-throughput screens, and the lead compounds that emerge can be optimized by combinatorial chemistry, or, in economically less privileged countries, by traditional clinical chemistry approaches.

Admittedly, drug development strategies based on naturally derived candidate compounds may present a considerable number of obstacles, which are not posed by those using rational synthesis. There may be problems of procurement due to inaccessibility of collection sites, difficulties with their isolation and production of the pharmacologically active ingredient, and serious legal disputes among governments about intellectual rights properties. Even so, screening of natural products seems more likely to yield a hit when compared with screening of rationally designed compounds.

Plant-derived compounds were also of great significance to cancer therapy. It was, for instance, only upon the addition of the Vinca alkaloid vincristine or oncovin (isolated from Catharanthus roseus, Apocynaceae) to mechlorethamine, prednisone, and procarbazine (the MOPP regimen) that the first cures in a human cancer (Hodgkin's disease) was achieved. The combination of the epipodophyllotoxin etoposide (derived from the mandrake plant Podophyllum peltatum and the wild chervil P. emodi, Berberidaceae), bleomycin, and cisplatin are highly active and curative regimen in testicular
cancer. Etoposide is furthermore one of the most active agents against small cell lung carcinoma(21).

Natural Products and Defense against Carcinogenesis:

A major group of natural products are the powerful antioxidants, others are phenolic in nature, and the remainder includes reactive groups that confer protective properties. These natural products are found in; plant extracts (Table 1) vegetables and fruits (Table 2). Although the mechanism of the protective effect is unclear, the fact that the consumption of fruit and vegetables lowers the incidence of carcinogenesis at a wide variety of sites is broadly supported. A host of plant constituents could be responsible for the protective effects, and it is likely that several of them play a role under some circumstances. Most of the non nutrient antioxidants in these foods are phenolic or polyphenolic compounds, such as iso flavones in soybeans, catechins in tea, phenolic esters in coffee, phenolic acid in red wine, quercetin in onions, and rosmarinic acid in rosemary.

Of the many anti carcinogens already detected in plant foods, the antioxidants vitamins C and E and the pro vitamin β-carotene have received the most attention(22). Although there has been considered enthusiasm for the potential anti carcinogenic properties of β-carotene, research findings suggest that several different carotenoids are likely to be associated with reduced cancer risks. In two intervention trials to investigate the potential protective effects of β-carotene against cancer, an unexpected significantly higher incidence of lung cancer was found in men taking supplements compared with those not taking additional β-carotene. These men were long-time heavy smokers and may represent a special case in that their lung cancer may have been initiated many years before the study took place(23). These results cause concern and need serious consideration. They do not invalidate the concept of the importance of antioxidant nutrients but do underline the need to examine the relative influence of supplements of a single antioxidant nutrient. (as distinct from complex mixtures of antioxidants in foods) as well as interactions between the effects of smoking, antioxidant nutrients, and disease progression.
Table 1: List of Plants Used as Anticancer Agents

<table>
<thead>
<tr>
<th>Source</th>
<th>Active component</th>
<th>Mechanism of action</th>
<th>Cancer inhibited (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizoma zedoariae</td>
<td>β- Elemene</td>
<td>Cell cycle arrest from S to G2M phase</td>
<td>24-25</td>
</tr>
<tr>
<td>Azadirachta indica Juss (Neem leaf)</td>
<td>Polyphenolic</td>
<td>Cytotoxic</td>
<td>Various cancers (26)</td>
</tr>
<tr>
<td>Muscadeneberries</td>
<td>Resveratrol</td>
<td>Antioxidant</td>
<td>Lung tumor in A/J mice (27)</td>
</tr>
<tr>
<td></td>
<td>myo-Inositol, dexamethasone</td>
<td>Antioxidant</td>
<td>Lung tumor in A/J mice, liver cancer (27, 28)</td>
</tr>
<tr>
<td>Curcuma longa L. turmeric</td>
<td>Curcumin</td>
<td>Antioxidant</td>
<td>Prostate, lung tumor in A/J mice (27, 29, 30)</td>
</tr>
<tr>
<td></td>
<td>Esculetin</td>
<td>Antioxidant</td>
<td>Lung tumor in A/J mice (27)</td>
</tr>
<tr>
<td>Cylopia intermedia (honeybushtea)</td>
<td>Polyphenolic compounds</td>
<td>Antioxidant, antimutagenic, interferes</td>
<td>Various cancers (31)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>With P450-mediated metabolism</td>
<td></td>
</tr>
<tr>
<td>Undaria pinnatifida (seaweed)</td>
<td>(Viva-Natural)</td>
<td>Prophylactic</td>
<td>Lewis lung cancer in mice (32)</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Constituents</td>
<td>Biological Activity</td>
<td>Tumor Models</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>--------------------------------------------------</td>
<td>----------------------------------------------------------</td>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>Scutellaria radix, S. indica</td>
<td>Valepotriates, Flavonoids, Isoflavones, Genistein (piperazine complex)</td>
<td>Cytotoxic Prostaglandin E2 production Protein tyrosine kinase inhibitor, diverse EGFR and p21 ras expression phenol types, dependent on epidermal cell growth factor receptor, estrogen-like action</td>
<td>(33) RatC6 glioma cells (34) Jurkat T-leukemia cells, bladder cancer (35-38)</td>
</tr>
<tr>
<td>Various plants</td>
<td>Quercetin, kaempferol, rutin, hesperidin</td>
<td>OH scavenger</td>
<td>B16 melanoma (39, 40)</td>
</tr>
<tr>
<td>Camellia sinensis, greentea, black tea</td>
<td>Polyphenols, epigallocatechin-3-gallate</td>
<td>Apoptosis induction, cell cycle arrest</td>
<td>Tumor cells (41, 42)</td>
</tr>
<tr>
<td>Coriolus versicolor (Chinese herb)</td>
<td>Bis-benzyl isoalkaloids, bufalin, berberine, tetrandrine</td>
<td>Apoptosis induction, complexes With DNA</td>
<td>HL-60, U937 cells (43-45)</td>
</tr>
<tr>
<td>Uncariatomentosa</td>
<td></td>
<td>Apoptosis induction</td>
<td>Tumor cells (46)</td>
</tr>
<tr>
<td>Eucalyptus grandis</td>
<td>Euglobal-G1</td>
<td>Apoptosis induction</td>
<td>Various cancers (47)</td>
</tr>
<tr>
<td>Ornithogalum</td>
<td>Cholestane glycoside</td>
<td>Apoptosis induction</td>
<td>HL-60 cells (48)</td>
</tr>
</tbody>
</table>
## Table 2: List of fruits and vegetables Used as Anticancer Agents

<table>
<thead>
<tr>
<th>Source</th>
<th>Active component</th>
<th>Mechanism of action</th>
<th>Cancer inhibited (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apples</td>
<td></td>
<td>Antioxidant</td>
<td>Various cancers (49)</td>
</tr>
<tr>
<td>Yellow-orange Vegetables and fruits</td>
<td>β-Carotene</td>
<td>Antioxidant</td>
<td>Various cancers (50, 51)</td>
</tr>
<tr>
<td>Carrots</td>
<td>α-and β -carotene, phenolic compounds</td>
<td>Antioxidant, pS2 gene expression, α - carotene more effective, inhibits tumors in rats and mice</td>
<td>Pancreatic, colon, breast cancer; liver cells; rat, Mice colon and liver cancer (49, 52-54)</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>Lycopene, vitamin C</td>
<td>Strong antioxidant, inhibits Lymphocyte DNA-oxidated damage</td>
<td>Leukemia, lung cancer; mice tumors (27, 55-57)</td>
</tr>
<tr>
<td>Citrus fruits</td>
<td>β -Cryptoxanthin, bioflavonoids, chalcones, vitamin C</td>
<td>Antioxidant, stimulates expression of RBgeneandp73gene (ap53-relatedgene)</td>
<td>Rat tumors; various cancers (54)</td>
</tr>
<tr>
<td>Garlic, onions, leeks, chives</td>
<td>Allicin, flavonoids, Vitamin C, selenium, sulfur</td>
<td>Detoxifies carcinogen, inhibits Helicobacter pylori, cell cycle Arrest from S to G2 M boundary phase</td>
<td>Stomach cancer (25, 58)</td>
</tr>
</tbody>
</table>
A number of naturally occurring compounds from vegetables and herbs exert chemo preventive properties against carcinogenesis. Most studies appear to test the natural products on human leukemia cells. The Chinese medicinal herb Rhizoma zedoariae, for example, produces a compound called lemele, which has been shown to exhibit antitumor activity in human and murine tumor cells in vitro and in vivo(25).

1.3.2 Mechanisms of Action of Natural Products on Carcinogenesis

In the last decade, advances in cancer research have enhanced our understanding of cancer biology and genetics. Among the most important of these is that the genes that control apoptosis have a major effect on malignancy through the disruption of the apoptotic process that leads to tumor initiation, progression, and metastasis. Therefore, one mechanism of tumor suppression by natural products may be to induce apoptosis, thereby providing a genetic basis for cancer therapy by natural products. The p53 protein, encoded by a tumor suppressor gene, mediates growth arrest or apoptosis in response to a variety of stresses. P53-Dependent apoptosis, occurring in several sensitive tissues after radiation or chemotherapy, is partially responsible for the side effects of cancer treatment, making p53 a potential target for therapeutic suppression. Hypoxic stress, such as DNA damage, induces p53 protein accumulation and p53-dependent apoptosis in oncogenically transformed cells. Unlike DNA damage, hypoxia does not induce p53-dependent cell cycle arrest, suggesting that p53 activity is differentially regulated by these two stresses. Genotoxic stress induces both kinds of interactions, whereas stresses that lack a DNA damage component, as exemplified by hypoxia, primarily induce interaction with cosuppressors. However, inhibition of either type of interaction can result in diminished apoptotic activity. Germ line mutations of the p53 tumor suppressor gene in patients with a high risk for cancer inactivate the p53 protein (59). Lung-specific
expression of the p53 and K-ras genes in mice was reported by Witschi et al. (28), Brockman et al. (60) & Wattenberg and Estensen (61), when mice were exposed to natural products, such as myo-inositol, dexamethasone, curcumin, esculetin, resveratrol, lycopene, and butylated hydroxyanisole. The question whether any of the known natural products modulate expression of the p53 protein requires experimentation(62).

Carcinogens in the diet that trigger the initial stage include moulds and aflatoxins (for example, in peanuts and maize), nitrosamines (in smoked meats and other cured products), rancid fats and cooking oils, alcohol, and additives and preservatives. A combination of foods may have a cumulative effect, and when incorrect diet is added to a polluted environment, smoking, UV radiation, free radicals, lack of exercise, and stress, the stage is set for DNA damage and cancer progression. In addition to the usual vitamin and mineral supplements, amino acids such as cysteine and natural antioxidants such as clove oil constituents are particularly helpful in offsetting problems caused by a variety of environmental toxins. Many diseases, including cancer, have been shown to be linked to a poorly functioning liver detoxification system. A study at an Italian chemical plant showed that workers with an inadequate liver detoxification enzyme later developed bladder cancer. Herbs that promote a healthy liver function include dandelion (taraxacum), milk thistle (silybum), and artichoke (cynara). Beetroot is particularly beneficial and may be eaten raw, cooked, or in juices. Raw vegetable juices, which may include carrots, celery, and parsley, together with beetroot are an excellent way of providing concentrated antioxidants and plant enzymes(51). Wheat grass is also useful. A diet rich in cruciferous vegetables and vitamins B (in whole grains and cereals) and C (cabbage, broccoli, and brussel sprouts) promotes liver detoxification. Other vitamin C foods are peppers, tomatoes, oranges, and tangerines. Glutathione-rich
foods, such as avocados, asparagus, and walnuts, are also good for liver detoxification. The current trend to identify natural products as new cancer preventative agents is based on a conceptual basis and understanding of their mechanisms of action in carcinogenesis (63).

The biological activities of medicinal plants are attributed mostly to their bioactive chemicals. Examples of these include some minerals such as selenium and many secondary metabolites. The secondary metabolites are compounds that are non-essential to the nutrition of plants, but are vital for the viability of plants producing them. They are usually produced in relatively small amounts. They serve a specific function at a certain developmental stage and are, therefore, expressed at various levels in the different phases of the plant's life. Many of the secondary metabolites, such as resveratrol in red grapes and quercetin in onions, possess protective effects against environmental pathogens.

Other bioactive compounds may play roles in protection against environmental stress, rapid healing upon plant injury, coloration of flowers and emission of scents to attract insects and involvement in formation of various structures like the cell wall. Secondary metabolites or phytochemicals are synthesized via three major metabolic pathways. The isoprenoid pathway produces terpenes, saponins and carotenoids. The cinnamic acid pathway produces flavonoids, isoflavonoids, coumarins and lignans. The third major pathway is the shikimik acid pathway, which is another pathway for the synthesis of phenolic compounds, particularly tannins (64). A considerable amount of research has been devoted to elucidating the molecular mechanisms by which herbal products inhibit cancer and inflammation (65).
Electrochemical Study of Anticancer Drugs:

Electrochemistry has been used in cancer pharmacology in a variety of ways. This is the area where the majority of studies of correlation between electrochemical parameters and biological activities were performed.

Electrochemistry is the branch of chemistry that deals the relationship between electricity and chemical changes. Of principal interest are the reactions that take place between electrodes and the electrolytes in electric and electrolytic cells as well as the reactions that take place in an electrolyte as electricity passes through it. In addition to analytical determinations, the electrochemistry has also gained reorganization and acceptance for its valuable applications in biochemistry, biotechnology, pharmacy, toxicology, engineering and electrochemical synthesis.

Organic electrochemistry is a branch of electrochemistry which deals with the study of elucidation/confirmation of structure, study of transient intermediates, nature of electron transfer process, initiation of polymerization, study of biological redox system and development of chemical sensor and biosensors. It is therefore important to undertake challenges and opportunities available in the field of organic electrochemistry. It involves the study of the chemical reactions that take place when an electric current is passed through a solution containing one or more organic compounds. It is a highly interdisciplinary science.

Organic electrochemistry has been of increasing interest for industrial applications in recent years because the costs of electrochemistry have been rising more slowly than the costs of conventional chemical reagents and electrochemical procedures can be environmentally less intrusive than other
chemical processes. In general, electrochemistry has been used in processes where bio-reduction or bio-oxidation is concerned. They can be classified in common theoretical frameworks or as analytical tools to observe, prove and predict biological phenomena. In spite of the division into classes of drugs’ mechanism of action, it should be emphasized that several factors may be operating in a multifaceted attack.

Electrochemical studies should furnish an enormous amount of evidence regarding the mechanisms of biological electron-transfer processes. Electro analytical techniques mainly polarography, cyclic, square wave and differential pulse voltammetry, coulometry, together with electron spin resonance (ESR) experiments are well developed methods for the drug analysis of samples with great accuracy and precision of determination up to nano-gram level in general and Pico-gram levels in favorable cases.

One obvious application of electrochemistry is related to the electro analytical studies of endobiotics and drugs, for quantification in biological liquids or other purposes. The presence of an electro active group or its transformation from electro inactive ones is a pre-requisite. Electrochemical detectors for analytical methods, such as differential pulse polarography or biosensors have been extensively used and play important role in endobiotics analyses(66).

Modern version of electro analytical techniques is used know a days for the purpose of drug analysis of substances in sample obtain from Biological, industrial environmental medicinal and natural origins. Advanced versions of Voltametry/ Polarography methods are used in the analysis of organic compound in different environment because these methods are non-destructive ones.
Polarography:

J. Heyrovsky invented the original polarographic method, conventional direct current polarography (DCP), and Heyrovsky and Shikata constructed the first polarograph in 1925. It has been defined as "A branch of electro analytical chemistry which deals with the measurement and interpretation of current voltage relationship during the electrolysis of a substance between two electrodes, one of which is very small. It essentially involves electrolysis at a microelectrode leading to the reduction or the oxidation of organic species.

Direct Current Polarography (DCP):

DCP involves the measurement of current flowing through the dropping mercury electrode (DME) as a function of applied potential. Under the influence of gravity, mercury drops grow from the end of a fine glass capillary until they detach. Then the process is allowed to repeat itself. Drops may be allowed to fall naturally or may be dislodged after a specified interval with the aid of a mechanical device. A major advantage of the DME is that a constantly renewed electrode surface is exposed to the test solution so that problems of electrode blockage are avoided. Another advantage of the DME is that it allows a number of electrode reduction processes to be monitored, which would otherwise be inaccessible, because a wide negative potential region is available on account of the high over potential for water reduction.

If an electro active species is capable of undergoing a redox process at the DME, then an S-shaped current-potential relation is usually observed. This is called a polarographic wave. Figure 1.1 illustrates the response obtained from a reduction reaction where the current (i) increases over a particular potential (E) range until it reaches a limiting value. The limiting current is the diffusion-controlled limiting current (i_d). This i_d is of interest in analytical measurements as it is proportional to the concentration of reactant. For a charge reaction

\[ A + ne = B \]
Ilkovic first put the measurement of this current on a theoretical basis, and his equation is (67-69).

\[ i_d = \left( \frac{7}{3} \right)^{1/2} (36 \text{ p})^{1/6} r^{2/3} \text{ nF} \ D^{1/2} \ m^{2/3} \ t_d^{1/6} \ C \]

where \( r \) is the density of mercury, \( n \) is the number of electrons, \( F \) is Faraday's constant, \( D \) is the diffusion coefficient, \( m \) is the flow rate of mercury, \( t_d \) is the drop time, and \( C \) is the concentration of the electro active species in the bulk solution.

A faradaic current whose magnetic is controlled by the rate at which an electroactive species diffuse found an electrode solution interface is known as diffusion current \( I_d \). It is proportional to the concentration of electroactive species.

\[ I_d \propto C \]

The potential of a polarographic (indicator) electrode at the point on the rising part of polarographic wave, were the difference between the total current and the residual current is equal to one-half of the limiting current is known as half wave potential which is denoted as \( E_{1/2} \).

Fig 1.1 Diagram of DC Polarogram, the limiting current \( i_l \), the residual current \( i_r \) and the half-wave potential \( E_{1/2} \)
Differential Pulse Polarography:

Differential pulse polarography (DPP) was designed by arranging a charging current of smaller magnitude, and by producing a peak-shaped I-E curve.

The potential-time waveform used in DPP is shown of Figure 1.2. A voltage ramp is applied to the electrode as in the DCP, and small amplitude potential pulse is added to the voltage towards the end of each drop's life. Two currents are measured before applying the pulse and at the end of the pulse. When the difference between the two current samples is plotted as a function of the applied ramp voltage, a peak-shaped current response is shown. In DPP the maximum value of faradaic current is due to the electro reduction of substances during the single potential is called peak current \( I_p \).

\[ I_p \alpha C \]

(C is the concentration of electrolyte species)

In the potential of working (indicator) electrode at which peak current is attained known as peak potential \( E_p \).

The peak-shaped I-E curve allows polarographic responses in close proximity to each other to be more clearly resolved than in either DCP. The detection limit s as low as \( 10^{-8} \) M can be achieved using DPP(70).

The factors which are effect the polarographic results are charge current, resistance, noise, convection, pH, the reactant number, and product numbers, standard redox potentials, rate of electron transfer, transfer coefficient, concentration, diffusion coefficient, forward and reverse chemical reaction rate constants, temperature, electrode area, and experimental parameters, etc.
Bioinorganic Study of Anticancer Drugs:

Bioinorganic chemistry is a specialized field that spans the chemistry of metal-containing molecules within biological systems. This field is concerned with the control and use of metal ions in biochemical processes. Although bioinorganic chemistry includes the study of artificially introduced metals (e.g., medicinally), many natural occurring biological processes (such as respiration) depend upon molecules containing inorganic elements, such as metalloproteins, and these natural processes are also studied by bioinorganic chemistry. Bioinorganic chemistry has developed from the continuing research in inorganic chemistry and its important associations in biological chemistry. As a mix of biochemistry and inorganic chemistry, bioinorganic chemistry is important in realizing the implications of electron-transfer proteins, substrate bindings and activation, atom and group transfer chemistry as well as metal properties in biological chemistry.

This field involves the application of the principles of inorganic chemistry to problems of biology and biochemistry. Because most biological components
are organic, that is, they involve the chemistry of carbon compounds; the combination of the prefix bio- and inorganic may appear contradictory. However, organisms require a number of other elements to carry out their basic functions. Many of these elements are present as metal ions that are involved in crucial biological processes such as respiration, metabolism, cell division, muscle contraction, nerve impulse transmission, and gene regulation. The characterization of the interactions between such metal centers and biological components is the heart of bioinorganic chemistry. Metal ions influence biological phenomena by interacting with organic functional groups on biomolecules, forming metal complexes (71). From this perspective, much of bioinorganic chemistry may be considered as coordination chemistry applied to biological questions.

Metal complexes have also been found to be useful as therapeutic or diagnostic agents. Prominent among metal-based drugs is cisplatin, which is particularly effective in the treatment of testicular and ovarian cancers. Gold, gallium, and bismuth compounds are used for the treatment of rheumatoid arthritis, hypercalcemia, and peptic ulcers, respectively. In clinical diagnosis, metal complexes can be used as imaging agents. The convenient half-life and radioemission properties of technetium-99 make its complexes very useful for a number of applications; by varying the ligands bound to the metal ion, diagnostic agents have been developed for imaging the heart, brain, and kidneys. Complexes of paramagnetic metal ions such as gadolinium (III), iron (III), and manganese (II) are also used as contrast agents to enhance images obtained from magnetic resonance imaging (MRI). There are several distinct systems of interest in bioinorganic chemistry. These areas include metal ion transport and storage, metallohydrolase enzymes, metal-containing electron transfer proteins, oxygen transport and activation proteins, bioorganometallic systems such as hydrogenases and alkyltransferases, and enzymes involved in nitrogen metabolism pathways.
Oxygen transport and activation proteins in human body make extensive use of metals such as iron, copper, and manganese. Hem is utilized by red blood cells in the form of hemoglobin for oxygen transport and is perhaps the most recognized metal system in biology. Other oxygen transport systems include myoglobin, hemocyanin, and hemerythrin. Oxidases and oxygenases are metal systems found throughout nature that take advantage of oxygen to carry out important reactions such as energy generation in cytochrome C oxidase or small molecule oxidation in cytochrome P450 oxidases or methane monooxygenase. Some metalloproteins are designed to protect a biological system from the potentially harmful effects of oxygen and other reactive oxygen-containing molecules such as hydrogen peroxide. These systems include peroxidases, catalases, and superoxide dismutases. A complementary metalloprotein to those that react with oxygen is the oxygen evolving complex present in plants. This system is part of the complex protein machinery that produces oxygen as plants respire.

In general, bioinorganic chemists tackle such problems by first focusing on the elucidation of the structure of the metal complex of interest and then correlating structure with function. The attainment of solutions usually requires a combination of physical, chemical, and biological approaches. Biochemistry and molecular biology are often used to provide sufficient amounts of the system for investigation. Physical approaches such as crystallography and spectroscopy are useful in defining structural properties of the metal site (72). Synthetic methods can be used for the design and assembly of structural, spectroscopic, and functional models of the metal site. All these approaches then converge to elucidate how such a site functions. Polarography is the one of the oldest but consistent inorganic analytical tool for the study of stoichiometry and formation constant of Metal-ligand complexes(73).
Metal Ligand Stochiometric Study by Polarography:

In the last two decades polarography has found increasing application as a means of identifying the stoichiometry and formation constants of co-ordination compounds when metal ion in solution undergoes complexation with ligand, their polarographic reduction waves are altered in two ways, firstly the half wave potential is shifted to more electronegative value in most of the cases. Second, the diffusion current changes and usually becomes smaller. Method for determination of stoichiometry and formation constants of metal complexes polarographically may be divided in to following categories.

1. Lingane’s method designed to study formation of a single complex species.

2. Deford-Hume method designed to study formation of plural complex species.

3. Shapp and mcMaster’s method designed to study mixed ligand complex formation of a metal with more than one ligand.

A simple and reasonable method has been designed by lingane for the study of metal ligand equalibria in cases where only one complex species is formed over the entire range of ligand concentration. Thus for a reversible reduction of a complex, compound formed by the reaction.

\[ M + PX \rightarrow MX_p \] (Ionic charge have been ignored for the simplicity).

The plot of change in half wave potential \( \Delta E_{1/2} \) vs. change in logarithm of ligand concentration \( \Delta \log C_x \) should be linear with a slope value of \(-PX\) 0.0591/n the stoichiometry of the complex is determined from the value of P for metal complex of the type MXp.
The dissociation constant can be determined by the following equation. 

\[(E_{1/2})_c - (E_{1/2})_s = 0.0591/n \log K_c - 0.0591/n \log C_x\]

Where, \((E_{1/2})_c\) = half wave potential of complexed species at ligand concentration \(C_x\).

\((E_{1/2})_s\) = half wave potential of the simple metal ion.

\(K_c\) = Dissociation constant of the complex species.

\(C_x\) = concentration of ligand (in moles).

\(n\) = Number of electrons involve in the electrode process.

\(P\) = Number of ligand molecules that are coordinated to one metal ion.

The above method is applicable only if the pure metal ion and its complex understudy is reversible reduced at the dropping mercury electrode and reduction is diffusion controlled.

**Amperometric Titration:**

The term amperometric titration was introduced by Kolthoff (1939) and co-workers. This name is used extensively in polarographic titrations in which current serves an indicator. Amperometric sensors exploit the use of a potential applied between a reference and a working electrode, to cause the oxidation or reduction of an electroactive species; the resultant current is measured (74). A change in the concentration of the substance to be determined is immediately reflected in the value of limiting current. By measuring the current (at a constant potential of the dropping mercury electrode in the region of limiting current), after each addition of the titrant and plotting the limiting current as a function of reagent added, amperometric curves are obtained.

In amperometry, potential applied across the indicator electrode and reference electrode is kept constant at the plateau potential value of either titrate or titrant, and the current passing through the cell is measured and plotted against
the volume of reagent added. Some most common type of curves encountered in amperometric titrations are \( \| \) shaped, \( \| \) shaped, V shaped and \( \| \) shaped.

Some advantages of amperometric titrations are describe below-

A. The titration can usually be carried out rapidly, since the end point is found graphically. A few current measurements at constant applied voltage before and after the end point are sufficient.

B. A number of amperometric titrations can be carried out at dilutions as low as \( 10^{-4} \) M and in favorable cases to \( 10^{-6} \) M. The range and sensitivity are higher than potentiometric and conductometric titrations.

C. Foreign salts may frequently to present without interference and are usually added as supporting electrolyte in order to eliminate the migration current and the results of the characteristics of the capillary.

D. The temperature need not be known provided it is kept constant during the titration.

Infrared Spectroscopy:

Infrared radiations are associated with much lower energy than visible and ultra-violet radiation and it is emitted as range of frequencies and energies from a hot body. IR provides valuable information about the nature of the co-ordinate bond, sites of attachment of the ligand and stereochemistry of the complexes. The donation of electron pair from the ligand to metal ion is generally accompanied by notable change in the position and intensity of the IR bonds of higher or lower wave number side takes place depending upon increase or decrease spectroscopy over the other usual methods of structural analysis is that.

This method can solve many problems in organic chemistry and co-ordination chemistry. The region of the electromagnetic spectrum known as the infrared begins at about 0.8 \( \mu \) and ends at about 500\( \mu \).
Antibacterial and Antifungal Activity:

Metal complexes play an important role in the biological activity of drugs. The complex formation has been suggested as one of the important mechanism of drug action. Since the dawn of human creation, it has been observed that microorganisms survive as saprophytes or parasites in nature. No one today, with a liveliest imagination can visualize, how devastating were the effects of bacterial infection.

In 1935 sulfonamide was first discovered and found effective in inhibiting growth of certain bacteria. Bacteria (Greek, bakterion- small staff) are for the most part unicellular organism lacking chlorophyll.

Since then scientist have been in search of antibiotics, which may be used to control the diseases, caused by microbes. The shape as well as the dimensions of microbes is not absolutely constant. Morphologically differences are found in many bacterial species.

Pharmacological Study:

The action of drug is determined not only by its chemical structure but also by its physicochemical properties. Any reaction in the body other than the desired response of a given drug is considered to be a toxic reaction. Ideally, a drug should be so safe that a therapeutic dose in every individual would produce the desired response and nothing more. Unfortunately none of the drugs developed in the past achieve this standard. Thus the toxicity of a drug must be studied before attending clinical use.

3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) is a monotetrazolium salt, the reduction of which is one of the most frequently used methods for measuring cell proliferation and cytotoxicity(75). The cell viability MTT assay is a colorimetric metabolic assay based on mitochondrial dye
conversion to assess viability. Tetrazolium salt is used to assess the activity of various dehydrogenase enzymes where the tetrazolium ring is cleaved in active mitochondria, demonstrating the presence of living cells(76). The main advantages of the colorimetric assay are it’s rapidly and precision, and the lack of any radioisotope. We have used the assay to measure proliferative, lymphokines, mitogen stimulations and complement-mediated lysis.

Aim and Scope of Present Study:

Today research in the medical and biological science are being pursued all over the world with the view for achieving better understanding of cancer, its causes, symptoms diagnostic treatment and possible cure.

Looking at the multidimensional applications and versatility of modern polarographic technique, they have been used in the present work entitled ‘Bioinorganic and electrochemical study of some anticancer natural drugs and pharmaceutical formulation’ to analyzes various anticancer natural drugs of pharmaceutical and extracted samples.

Of late, study of drug complexes have assumed much importance particularly complexes of drugs with metal ions essential for life processes have drawn attention of a large number of chemists. The chemistry of life involves, in an essential and indispensable way, many of the chemical elements including metals. The importance of Na, Ca and Fe has long been recognized but many others especially Cu, Zn, Mn, Mo and Co are necessary for life. Cu and Zn are known to form metalloenzymes. The metal ion does not merely participate during the time that enzyme substrate complex exists, but is a permanent part of the enzymes.

Electrochemical study furnishes evidence regarding the mechanism of biological electron transfer processes. An up to date survey of relevant literature
reveals that the electrochemical and bioinorganic methods are in use to obtain relevant information about drug and its metal complexes.

The objective of the present work is two folds. It seeks to investigate the structure of the complex of certain very important anticancer natural drugs like Curcumin, Indole-3-Carbinol, Beta-carotene and Aloe-emodin with certain life essential metal ions viz Cu (II), Zn (II), Fe (II). It also aims to know the changes in the activities of drugs due to complexation with the proposed metals.

Besides, the present investigation also incorporation the qualitative and quantitative analysis of these drugs in extracted and pharmaceutical formulations using polarography (DCP, DPP) methods. The IR spectroscopy has been used to ascertain the metal ligand binding sites and the quantitative estimation of drugs was made possible by amperometric method.

Antimicrobial activity of these complexes with gram positive and gram-negative bacteria and Fungi has also been extensively studied.

It would be possible in favorable cases, where the potency of drug-metal complex is more than the parent drug, to recommend the drug–metal complexes in lieu of the respective drug for the therapeutic uses. However, it would be possible in case of some drugs to explain the therapeutic failure of the drug in a particular segment of population, which may contain an increased level of particular metal in-vitro due to environmental exposure.
References:


