CHAPTER 2

2.0 REVIEW OF LITERATURE

2.1 INDIAN MEDICINAL PLANTS WITH ANTIOXIDANT ACTIVITY

The traditional medicine that was practiced in India and all over the world is nowadays revalued by an extensive activity of research on different plant species and their therapeutic principles. Experimental evidences suggest that free radicals and reactive oxygen species can be involved in a large number of chronic age related diseases [2,3]. As the Indian medicinal plants produce a lot of antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new phytochemical compounds with potent antioxidant activity. Ayurveda, the Indian traditional health care system (ayus means life, Vedameans knowledge) means science of life. Ayurveda is the oldest medical system all over the world and is now being revived in its complete form under the name of Maharishi Ayurved [4].

The World Health Organization has approved of its richness and efficiency [5]. This system of traditional medicine provides an approach to the
prevention and treatment of different diseases by a large number of elaborate medical procedures and explicit pharmaceuticals. One of the major clinical specialties of Ayurveda is Rasayana. Rasayana is not only a drug therapy but is also a specialized procedure that was practiced in the form of rejuvenating recipes and dietary regimen and thus promoting good habit. The purpose of Rasayana is two-fold, namely, prevention of disease and counteract of aging process which result mainly from optimization of homeostasis. The meaning of the word Rasayana (rasa means essence, water; ayana means going) essentially refers to nutrition and its acquisition, movement, circulation and perfusion into the different body tissues [6]. Sharma et al.[7] has reported that the Rasayana drug therapy is mainly due to the strong antioxidant activity of any Rasayana. These Rasayana compounds were found to be thousand times more potent than ascorbic acid, α-tocopherol, and probucol.

2.2. ETHNO PHARMACOLOGY OF THE MEDICINAL PLANTS

Many studies and evaluations have been performed to identify antioxidant phytochemical compounds with a real pharmacological activity and a limited toxicity. In this context, ethno pharmacology represents the most important way by which it is possible to discover interesting and therapeutically useful bioactive compounds. The phytochemical analysis of Rasayana has revealed a large number of phytochemical compounds including tannic acid, flavonoids, tocopherol, curcumin, ascorbic acid, carotenoids, and polyphenols etc. which have been evaluated to contain potent antioxidant properties [21,22]. The herbal mixture preparations of Indian traditional medicine may have potent antioxidant activity arising from their content of plants with active antioxidant principles that act probably in a synergistic way. This hypothesis along with the lack of toxicity [23] can be an important guide
to understand and evaluate their wide use in the ancient period as well as in the present days.

Cancer chemoprevention by using natural antioxidant constituents has been suggested to offer a good potential in providing important fundamental benefits to public health and is now considered by many physicians and researchers as a key strategy for inhibiting, delaying, or even reversal of the process of carcinogenesis [24,25]. Moreover, knowledge and application of such potential antioxidant activities derived from medicinal plant sources in reducing oxidative stresses in vivo has prompted many investigators to search for potent and cost effective antioxidants from various plant sources [26,27,28,29,30,31,32,33]. These research activities have contributed to new and renewed public interests worldwide in herbal medicines, health foods and nutritional supplements.

Reactive oxygen species (ROS) such as super oxide anions, hydrogen peroxide, and hydroxyl radicals, nitric oxide and peroxy nitrite radicals play a significant role in the generation of oxidative stress and stress related diseases and also to the pathogenesis of various important age related diseases [30, 31]. In healthy individuals, the production of the free radicals is balanced by the antioxidant defense system of the body. However, oxidative stress is generated when equilibrium favours the free radical generation which result as a consequence of a depletion of antioxidant levels. The oxidation of lipid, DNA, protein, carbohydrate and other biological molecules present in the cells or tissues by toxic ROS may cause DNA mutation and serve to damage the target cells or tissues. This often results in cell senescence and death of the damaged cells or tissues.

Botanicals have used the medicinal plants for treatment and prevention of various human diseases throughout history. The cancer chemo preventive activities of naturally occurring phytochemical compounds are of great
interest. Many indigenous herbal plants of regional interest have been used popularly as folk medicines in India and also in other countries. However, their bioactivities or pharmacological effects have to be characterized and elucidated before it could be used in the treatment of diseases.

2.3. ANTIOXIDANT PROPERTIES OF TRADITIONAL MEDICINAL PLANTS

The use of traditional herbal medicine is widespread and plants still present a major source of novel active biological compounds with varied and versatile activities, including anti-inflammatory, anti-carcinogenic, anti-viral, anti-bacterial and cardio protective activities. Antioxidants can play a major role in executing the beneficial role of these health promoting activities [34]. The numerous beneficial effects attributed to phenolic products have now given rise to a new interest in finding medicinal plant species with high phenolic content and the relevant biological activity. Berries are rich dietary source of phenolic antioxidants and bioactive properties [35,36].

Antioxidant based drugs or pharmaceutical formulations that are prepared for the prevention and treatment of various complex chronic age related diseases such as atherosclerosis, stroke, diabetes, Alzheimer’s disease and cancer have gained importance in the past few decades [37]. This has attracted a great deal of research interest in the isolation and characterization of these natural antioxidants. Subsequently, a worldwide trend towards the use of natural antioxidant phytochemicals obtained from berry crops, tea, herbs, oilseeds, beans, fruits, and vegetables has increased in recent times [38,39,40].

Several herbs and spices have been reported to show antioxidant activity. Rosemary, sage, thyme, nutmeg, turmeric, white pepper, chilli, pepper, ginger, garlic and several medicinal plants extracts exhibit antioxidant activity [41,42,43,44]. The main active antioxidant compounds isolated from the medicinal plants include flavonoids, isoflavones, flavones, anthocyanins,
coumarins, lignans, catechins, and isocatechins. In addition to the above antioxidant phytochemical compounds, vitamins such as α- tocopherol, β-carotene and ascorbic acid also show antioxidant properties [45,46,47]. A direct relationship between the antioxidant activity and Phenolic content of the plant extracts has also been reported by many research works [47,48]. Epidemiological studies have shown that the consumption of food and beverages that are rich in phenolic content can reduce the risk of coronary heart disease and other related diseases [49]. Many Indian medicinal plants are considered potential sources of the above mentioned antioxidant compounds. The active constituents present in these medicinal plants are also being studied extensively.

2.3.1 Free Radical Scavenging Activity of Antioxidants

Reactive oxygen species (ROS) are an entire class of highly unstable and reactive molecules that are produced from the metabolism of oxygen. ROS which includes superoxide radicals, hydroxyl radicals and hydrogen peroxide are mainly produced as byproducts of thousands of biochemical reactions occurring on the body or from exogenous sources. In vivo studies have shown that some of these ROS play important physiological roles in maintaining the cell physiology. But at the same time they can also cause damages to cell membranes and DNA structures inducing oxidation in the cells. This causes cell membrane lipid peroxidation, decreased membrane fluidity and DNA mutations which ultimately lead to cancer and degenerative diseases [50,11,8,10].

Mammalian cells possess elaborate and extensive defense mechanisms for radical scavenging action. The crucial metabolic steps are catalyzed by superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) which destroy the toxic peroxides. In addition to these antioxidant enzymes, non-enzymatic molecules which include thioredoxin, thiols, and disulfide-bonding play important functions in antioxidant defense
mechanisms. Some of these compounds are exogenous and are obtained from food sources. These antioxidants include vitamins such as α-tocopherol, β-carotene and ascorbic acid and micronutrient elements such as zinc and selenium [51]. If the cellular mechanisms do not effectively scavenge these free radicals they can lead to various chronic disease conditions.

2.3.2 Biological Role of Antioxidants

Antioxidants refer to the substances that delay the oxidation process by inhibiting the polymerization chain reactions which are initiated by free radicals and also the other subsequent oxidizing reactions following it. [52]. This concept of antioxidant activity is basic and fundamental to all branches of biochemical, nutraceutical, food chemistry and phytochemical sciences. Many of the synthetic antioxidants like butylated hydroxy toluene (BHT) have been widely used to preserve the freshness and also the quality of food by protecting against many of the oxidation related deterioration reactions.

An explicit growing ocean of literature points to the importance of the action of natural antioxidants obtained from many plant and herbal resources. Such sources of antioxidants may be used to reduce the risk caused by cellular oxidative damage, not only in food that is taken through the diet, but also that is present in the human body. This will definitely provide protection against many of the chronic diseases, including cancer and other neurodegenerative diseases, inflammation and cardiovascular diseases [53].

This increasing interest in the measurement of the antioxidant activity of different medicinal plant samples is obtained from the overwhelming evidence of the important role played by Reactive Oxygen Species (ROS), which mainly includes the superoxide (O$_2^-$), peroxyl (ROO$^\cdot$), alkoxyl (RO$^\cdot$), hydroxyl (OH$^\cdot$), and nitric oxide (NO$^\cdot$) radicals in various aging and chronic diseases. Several biochemical methods have been developed to measure the antioxidant activity in biological samples, which mainly includes the oxygen
radical absorption capacity (ORAC), ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazil (DPPH) radical scavenging activity and inhibition of formation of thiobarbituric acid reactive species (TBARS) [53,54].

2.3.3 Antioxidants and Cancer

It has been estimated through intensive research work that every human cell is exposed to approximately 105 oxidative damages a day from the free radicals that are produced in the course of thousands of metabolic reactions occurring in the body. Reactive oxygen species are normal oxidant by products that are produced as a result of aerobic metabolism. Under normal metabolic conditions of the cell approximately 2–5% of O₂ that is consumed by the mitochondria is converted to Reactive oxygen species [55,56]. The oxidative stress that is produced permanently changes the genetic material of the cell and lead to a number of degenerative and chronic diseases such as cancer [57]. Misrepair of the DNA damages could result in mutations such as base substitution or deletion which could lead to carcinogenesis [58].

Two different mechanisms are shown to play an important role in leading to oxidative damage and in the development of carcinogenesis.

The first mechanism occurs through the modulation of gene expression. Epigenetic effects on the gene expression can result in the stimulation of growth signals and proliferation of cells [59]. Chromosomal rearrangements in the cell are believed to result from strand breakage or misrepair leading to genetic amplifications, iterations in gene expression and loss of heterozygous nature. This in turn may promote neoplastic progression [60]. Reactive oxygen species have been shown to stimulate protein kinase and poly (ADP ribosylation) pathways which in turn affects the signal transduction pathways. This further leads to modulation of the expression of essential genes for
proliferation and tumor formation [61]. The free radical signal may be mediated through *ras* signal transduction pathways [62].

In the second mechanism, the radicals induce genetic alterations like chromosomal mutations and rearrangements, which can play a vital role in the initiation of carcinogenesis [63,64]. Oxidative DNA damage can result in a number of chromosomal abnormalities. This causes a block in DNA replication and a wide cytotoxicity [60]. Mutations can result because of misrepair or due to incorrect replication. Chromosomal rearrangements can result from strand breakage misrepair. It is well known that repair mechanisms decay with age and therefore DNA lesions accumulate with progression of age [65]. The sequence specificity of the DNA damage sites affects the mutation frequency [66]. Therefore, investigation of the sequence specificity of DNA damage would be beneficial for prevention of cancer. Thus mutagenic potential is directly proportional to the number of oxidative DNA damages that escape repair mechanisms.

The extraction, characterization and utilization of natural antioxidants from medicinal plants that may serve as potent source in combating carcinogenesis and aging process which are in progress [67,68].

2.3.4 Safety Concerns of Synthetic Antioxidants:

Synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiarybutylated hydroquinon (TBHQ) and gallic acid esters have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. These synthetic antioxidants also show low solubility and moderate antioxidant activity [69,70].
Many synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as antioxidants since hundreds of years. But the results of toxicological studies which have linked some synthetic antioxidants to the development of cancer and other diseases and have forced regulatory agencies to impose restrictions on their use in human foods [71,72,73,74]. Therefore, use of BHA is no longer permitted in food preparation in Japan and also in a number of other countries. Moreover, TBHQ is banned in Canada, Japan and European countries [75]. The public health concern on chemical additives and food safety measures has stimulated a continuing research for antioxidants derived from plant sources.

2.3.5 The Need for Natural Antioxidants Replacing Synthetic Antioxidants

Increasing health concern has now made the natural antioxidants replace synthetic antioxidants in food preparations. For instance, sage, mace and black pepper have been used to inhibit the oxidation process in frozen meat [76]. Galangal and rosemary extracts have been found to contain significant antioxidant properties. The antioxidant property of Galangal was found to improve the oxidative stability and also extended the shelf-life of minced beef [77]. The antioxidant properties of rosemary possess the ability to inhibit the formation and decomposition of hydro peroxides in corn oil and corn oil-in-water emulsions [78]. Tomato powders were identified to inhibit the singlet oxygen-catalyzed oxidation of linolenic acid and also copper-induced lipid peroxidation [79]. In addition, plants such as turmeric, passion fruits, Swiss chards, Canadian prairies, and many vegetables have also been studied for their potential use in food as natural sources of antioxidants [80,81,82]. Tea contains a very high concentration of polyphenols. Extracts of tea have become commercially available in recent years as antioxidants to control oxidation of lipids [83]. These natural antioxidants protect the DNA, protein, and membrane lipids of the cells from oxidative damage and
deterioration in biological systems and thus provide additional health benefits and prevent stress related diseases [84]. Phenolic compounds extracted from curcumin and clonal herbs have been extensively investigated for their potent medicinal application. They have been identified to possess very good antioxidant and anti-inflammatory properties [85,86,87]. More attention has been diverted to the human health promotion and the protective effects of plants due to their high content of natural antioxidants [88,75,89]. New antioxidants are highly in demand since antioxidants with suitable physicochemical properties are required for treating various diseases.

2.3.6 Mechanism of Antioxidation and Disease Prevention

Oxidation is a normal biochemical reaction occurring in the living cells where electrons are transferred from one atom to another. This results in the molecule which is losing an electron being oxidized. Free radicals are regenerated in the human body every day in this process when oxidation occurs during aerobic respiration. Reactive oxygen species (ROS) are nothing but the oxygen-centered free radicals. They exist in various forms like superoxide’s (O2•-), peroxyls (ROO•), alkoxyls (RO•), hydroxyls (HO•), and nitric oxides (NO•) [90] (Pietta, 2000). These free radicals are also derived from external sources such as cigarette smoke, charred food, air pollution, ultra-violet light and ionizing radiation. The free radicals present in the body not only causes premature aging and wrinkles, but they are also the primary cause of cancer and other age related chronic diseases. Fortunately, our body has been adapted with these hazardous changes that are taking place in the atmosphere over the years and it has now developed various types of defense mechanisms and biochemical changes to reduce the damage done by these free radicals.

Antioxidants are the main and primary defense mechanism present in our body acting as scavengers of free radicals that are being produced in the body as a result of thousands of chemical reactions[90]. Besides damaging the
vital living cells in the body and causing severe diseases, ROS can also potentially cause oxidation in food products and thus cause food spoilage. The major concern is primarily focused on oxidation of lipids, which alter the biochemical properties of food products by causing off-flavours, shortening the shelflife and diminishing the sensory aspects of food products. Antioxidants produced within the body include enzymatic antioxidants like dismutase, peroxidase, and catalase enzymes, as well as non-enzymatic antioxidants like glutathione (GSH) and cytochrome P450 [91]. Antioxidants derived from fresh fruits and vegetables include phenolic compounds, anthocyanins, carotenoids; vitamin C and vitamin E. Synthetic antioxidants which are commonly used as food additives include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (THBHQ). Regardless of the sources of antioxidants, all these different types of antioxidants have a similar function. Antioxidants prevent the damages caused by free radicals that are produced in the course of thousands of biochemical reactions occurring in the body every day.

There are basically three classes of antioxidant capabilities in the human body.

The first is work done by enzymes to control initial free radical production. When oxygen is taken in and used during aerobic respiration, superoxide, hydrogen peroxide, and hydroxyl radicals are all commonly formed [92]. Catalase and dismutase enzymes decrease the formation of hydroxyl radicals [93]. Glutathione peroxidases remove all peroxides, including hydrogen peroxides [94]. All the enzymes require metal co-factors to function. For example, dismutases require copper, zinc or manganese, depending on the pH [93] to function. On the other hand, catalases require iron as a co-factor, and glutathione peroxidases may require selenium [94].
The second class of antioxidants comes from the diet. Dietary antioxidants are found in various fruits and vegetables and medicinal plants. Dietary antioxidants are crucial in the human body because they are capable of ending the chain reaction of free radicals through proton donation.

The third class of antioxidants is replenishers. The source of protons comes from structures that can readily donate a proton, while remaining stable so as not to become a free radical. Examples of replenishers include carotenoids, flavonoids coenzyme Q, and glutathione. Both β-carotene and coenzyme Q work in lipid material and have a synergistic relationship with vitamin E. Glutathione and flavonoids are tied more to the aqueous environment where they can work both as proton donors to free radicals, and act to replenish vitamin C. Uric acid also contributes protons to antioxidants [95].

Other methods to control the impact of free radicals include the body’s handling of metals, and its ability to correct damage done through oxidation. When metals such as copper, iron, and manganese are not being used in the mitochondria to turn superoxide back into oxygen, or as co-factors to enzymes they are always bound to a carrier (example, iron has ferritin or transferrin). They would be far too catalytic in the formation of new free radicals if not bound because of their ability to donate and accept an electron.

Antioxidants have the ability to repair damage done by free radicals and this is thought to reduce cancer risk and aging. Repair of DNA is done by glutathione where this antioxidant donates protons to mildly damaged DNA. On the other hand, the repair of lipids is done by phospholipase enzymes, which catalyze the cleavage of peroxidized fatty acid side chains from the membrane, and replaces them with new, undamaged fatty acids [96]. In cases where free radical attack is uncontrollable and cell damage cannot be repaired, the end result would be growth of cancerous cells.
2.3.7 ANTIOXIDANT ACTIVITY
2.3.7.1 Determination of DPPH Radical Scavenging Activity

Several methods are used to evaluate antioxidant activities of natural compounds in foods or biological systems with varying results. The free radicals that are commonly used to assess antioxidant activity in vitro are 2, 2’-azobis (3-ethyl-benzothiazoline-6-sulfonic acid)(ABTS) and 2,2-diphenyl-1-picrylhydrazyl(DPPH). Other methods that are commonly used in the United State are the oxygen radical absorbance capacity (ORAC) assay and the ferric ion reducing antioxidant power (FRAP) assay.

The DPPH is a stable free radical with maximal absorption at 515 nm. It loses this absorption when reduced by an antioxidant or a free radical species [97]. The reaction mechanism is shown in the diagram below where AH is the antioxidant and R. is the free radical species:

\[
\text{DPPH}^* + \text{AH} \rightarrow \text{DPPH-H} + \text{A}^* \\
\text{DPPH}^* + \text{R} \rightarrow \text{DPPH-R}
\]

As mentioned above, the ORAC-FL assay is further extended to lipophilic antioxidants by using methylated β-cyclodextrin as a water solubility enhancer [98].

The DPPH method allows a direct investigation of the ability for the extractor antioxidant to donate hydrogen and/or electrons to quench the DPPH radical, DPPH. As the radical is quenched by antioxidants, the color of the solution changes from a deep purple to a light yellow and the absorbance at 515 nm decreases. The decrease in absorbance at a reaction time is used in determining the antioxidant activity of the tested substances with Trolox (2, 5,7,8-tetramethylichroman-2-carboxylic acid) as standard.
The DPPH method is widely used to determine antiradical/antioxidant activity of purified phenolic compounds as well as natural plant extracts. The antioxidant activity of sweet potato has been analyzed based on the DPPH method and the results suggested that the data was reproducible [99]. Cevallos-Casals and Cisneros-Zevallos [100] of Texas A&M University analyzed the antioxidant activity of red sweet potato and Andean purple corn using the DPPH method. Their results were promising as they indicated that the antioxidant activity of sweet potato was comparable to blueberries. This method also has good repeatability and is used frequently. However, the DPPH method also has its limitations. Bondet et al. [101] reported that most phenolic antioxidants react slowly with DPPH, reaching a steady state in 1-6 hours or longer. This suggests that antioxidant activity using DPPH should be evaluated over time. Furthermore, color interference of DPPH with samples that contain anthocyanins leads to underestimation of antioxidant activity [102]. Brand-Williams et al. [97] found that certain antioxidant compounds have different reaction kinetics with DPPH•. Antioxidants such as BHT and protocatechuic acid did not reach steady state, or the reaction endpoint, until three and two hours respectively, whereas compounds like ascorbic acid, isoascorbic acid, and isoeugenol achieved steady state within one minute.

At steady state, the DPPH• reaction has been shown to have a stoichiometric correlation with the quantity of antioxidant present. Caffeic acid, gentisic acid and gallic acid exhibited the highest antiradical activity with stoichiometry of 4.54, 5.6, and 6.25 reduced DPPH• molecules per molecule of antioxidant respectively, while one molecule of phenol, ascorbic acid, α-tocopherol, and BHT reduced <1, 1.85, 2, and 2.63 molecules of DPPH• respectively [97].

There are three ways to explain the different efficiencies of monophenolic compounds in reducing one DPPH•. One mechanism involves
the delocalization of an electron onto the parasubstituted OH group of the molecule prior to the donation of second hydrogen to reduce DPPH•. Another pathway involves the dimerization between two phenoxy radicals in which two hydroxyl groups would be regenerated through an intramolecular transfer of H•, consequently reacting further with DPPH•. The final pathway stated is a complexation of the aryl radical directly with the DPPH•. All of these pathways depend on the structure of the antioxidants themselves, whether it will go through a dimerization between antioxidants or a direct complexation with DPPH• depends on the stability and reaction potential of the molecular structure [97].

2.3.7.2 Determination of Total Phenolic Content

There are several methods available for analyzing the total phenol content in plant foods including precipitation with heavy metals, precipitation by the addition of organic compounds, oxidation under controlled conditions, and formation of colored products with various chemical elements. The very first method developed, the Folin-Ciocalteu method [103] was developed in 1927 and it originated from chemical reagents used for tyrosine analysis in which oxidation of phenols by a molybdotungstate reagent yields a colored product at 745-750 nm.

\[
\text{Na}_2\text{WO}_4/\text{Na}_2\text{MoO}_4 \rightarrow (\text{phenol-MoW}_{11}\text{O}_{40})^{-4}
\]

\[
\text{Mo(VI) (yellow)} + e^- \rightarrow \text{Mo(V) (blue)}
\]

The Folin-Ciocalteu procedure is based on the reductive power of aromatic hydroxyls with the phosphomolybdate complex of the Folin-Ciocalteu reagent (phosphomolybdate and phosphotungstate). This method measures the total hydroxyl groups of phenolic compounds. The absorbance is obtained by spectrophotometer reading at 725 nm. The concentration of the total phenolic content is determined by comparison with the optical density
values of different concentrations of a standard phenolic compound, either gallic acid or chlorogenic acid, and is expressed in terms of gallic acid or chlorogenic acid equivalent [104].

2.4 ANTIMICROBIAL ACTIVITY

Indian medicinal plants are potential sources of antimicrobial compounds. Infectious diseases are the second leading cause of death worldwide [105]. Treatment of microbial infections continues to be a problem in modern times because of severe side effects of synthetic drugs and growing resistance to antimicrobial agents. Hence, an extensive search for new, safer and more potent antimicrobials from medicinal plants is a pressing need. Herbal medicines have received much attention as a source of new antibacterial drugs since they are considered as time-tested and comparatively safe both for human use and also for the environment [105].

Clinical microbiologists have two important reasons to be interested in antimicrobial compounds from plant extracts. First, it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians. Several antimicrobial plant extracts have already been tested in humans. It is reported that, on average, two or three antibiotics derived from microorganisms are launched each year [106]. After a downturn in that pace in recent decades, the pace is again quickening as scientists realize that the effective life span of any antibiotic is limited. Worldwide spending on finding new anti-infective agents including vaccines is expected to increase 60% from the spending levels in 1993 [107]. New sources, especially medicinal plant sources, are being investigated. Second, the public is becoming increasingly aware of problems with the overprescription and misuse of traditional and conventional strong antibiotics. In addition, many people are interested in having more autonomy over their medical care. A multitude of plant compounds often of unreliable purity is readily available over the counter.
from herbalsuppliers and naturalfood stores, and self-medication withthese substances is common. The use of plant extracts, aswell as other alternative forms of medical treatments, has gained popularity in the late 1990s. Earlier in this decade,approximately onethird of people surveyed in the UnitedStates used at least one unconventional therapy during theprevious year [108]. It was reported that in 1996, sales of botanicalmedicines increased 37% over 1995 [109]. It is speculatedthat the American public may be reacting to overprescription of sometimes toxic drugsjust as their predecessors ofthe 19th century reacted to the overuse of bleeding,purging, and calomel [110].

2.5 WOUND HEALING ACTIVITY

Wounds are defined as physical injuries that occur in the body and result in an opening or break of the skin. Proper healing of the wounds or injuries isessential for the quick or proper restoration of the disrupted anatomical continuity and disturbed functional status of the skin [111]. Healing is a complex and intricate process initiated in response to an injury that helps to restorethe normal function and integrity of damaged tissues [112]. Wound healing process involvescontinuous cell–cell and cell–matrix interactions that allow the process to proceed in three overlappingphases,namely, inflammation (0–3 days), cellular proliferation (3–12 days) and remodeling (3–6 months) [113,114,115]. The process of healing of wounds requires the collaborative efforts of many differenttissues and cell lineages [116]. It mainly involves platelet aggregation and blood clotting, formation offibrin, an inflammatory response to injury, alteration in the ground substances, angiogenesis and re-epithelialization of the skin. Healing is not complete until the wounded and disrupted surfaces of the skin are firmly knit by collagen [117]. The basic principle of optimal and proper wound healing is to minimize tissue damage and provide adequatetissue perfusion and oxygenation, proper nutrition and moist wound healing environment to restore theanatomical continuity and function of the affected part [118].
In India, medicines based on medicinal plants and herbal origins have been the basis of treatment and cure for various diseases [119]. Moreover, Indian folk medicine comprises numerous prescriptions for therapeutic purposes such as healing of wounds, inflammation, skin infections, leprosy, diarrhea, scabies, venereal disease, ulcers, snake bite and many more diseases and disorders [120]. More than 80% of the world’s population still depends upon traditional medicines derived from plant sources for treatment of various skin diseases [121]. Herbal medicines in wound healing management involve disinfection, debridement and providing a moist environment to encourage the establishment of a suitable and favorable environment for natural wound healing process [122].

2.6 ANTIGENOTOXIC ACTIVITY

Ionizing radiations produce deleterious effects in the cells of all living organisms and the rapid technological advancement has increased human exposure to ionizing radiations enormously. Therefore, there is a need to protect humans against the hazardous effects of such ionizing radiations. Attempts are being devised to protect humans against the deleterious effects of such ionizing radiations by pharmacological intervention. Studies were made as early as 1949 and efforts are continued to search for radiation protectors, which may be of great help for human application. Many of the Indian medicinal plant studies mainly dwell on the antigenotoxic potential of medicinal plant and herbal extracts. In this study, an attempt has been made to appreciate the importance of the conceptual basis of these systems in evolving the material medic [123].

The results obtained from in vitro and in vivo studies indicate that several plants such as Gingko biloba, Centella asiatica, Hippophae rhamnoides, Ocimum sanctum, Panax ginseng, Podophyllum hexandrum, Amaranthus paniculatus, Emblica officinalis, Phyllanthus amarus, Piper
longum, Tinospora cordifoila, Mentha arvensis, Mentha piperita, Syzygium cumini, Zingiber officinale, Ageratum conyzoides, Aegle marmelos and Aphanamixis polystachya protect against radiation-induced lethality, lipid peroxidation and DNA damage. The fractionation-guided evaluation may help to develop new radioprotectors of desired activities.

2.6.1 The Need for Radio protectants

The discovery of X-rays by Roentgen in the year 1895 and radioactivity by Becquerel in the year 1896 can be considered as the turning point in human health care as the X-rays allowed peeping inside the human body [124,125,126]. Although harmful effects of ionizing radiations were reported within a few months of discovery of X-rays, the real magnitude was not known. Study of occupational workers like physicians and scientists handling radioactivity gave a clear picture of the harmful effects of ionizing radiations, which was further strengthened after the study of Japanese atomic bomb survivors of 1945. It is now fairly well established that radiation produces deleterious effects on the organisms and widespread use of radiation in diagnosis therapy, industry, energy sector and inadvertent exposure during air and space travel, nuclear accidents and nuclear terror attacks requires safeguard against human exposures. Lead shielding and other physical measures are cumbersome to use in such situations. Therefore, pharmacological intervention could be the most prudent strategy to protect humans against the harmful effect of ionizing radiations.

2.6.2 Chemical Radiation Protection

The use of chemicals to protect the harmful effects of radiation was attempted after World War II with the realization of the need to safeguard humans against the military use of atomic weapons. Patt and his co-workers[127] were the first to investigate the effect of amino-acid cysteine in rats exposed to lethal doses of X-rays [127]. They found that pretreatment of
rats protected them against the radiation-induced lethality. Thereafter, several chemical compounds and their analogues have been screened for their radioprotective ability. The high toxicity at optimum protective doses precluded their clinical use [128,129]. The other major drawback of these compounds was that they were unable to provide post-irradiation protection. With the recognition that normal tissue protection during radiotherapy is as important as the destruction of cancer cells, the focus of protection research became more therapy oriented [130]. Recent terror attacks throughout the world has strengthened the idea that it is necessary to devise appropriate measures against the nuclear terror attacks by using pharmacological agents that can protect against the ill effects of radiation.

The high toxicity of thiol compounds necessitated search for alternative agents, which could be less toxic and highly effective at non-toxic dose levels. It was also thought that products and phytochemical compounds isolated from natural sources could be of substantial use as non-toxic radioprotectors. Therefore, investigators diverted their attention towards the plant and natural products during the last two decades.

2.6.3 Assessment of Antigenotoxic Potential of Plants and Herbs

The most pragmatic approach to select the best possible and apt substance to evaluate antigenotoxic activity is to look into the available properties of the phytochemicals. Whether the phytochemicals have anti-inflammatory, antioxidant, antimicrobial, immunomodulatory, free radical scavenging or anti-stress properties and if it contains the above mentioned properties it may act as a potential radiation protector and could be the right phytochemical for evaluation of its antigenotoxic activity.

There are other short-term tests like DNA strand breaks, apoptosis and estimation of glutathione (GSH) and enzymes like catalase, glutathione peroxidase etc. that can also provide an inkling of the Radioprotective activity
of any pharmacological agent. However, the gold standard for radioprotective activity is the evaluation of 30-day survival in rodents, since the animal studies with death as the end point are the most confirmatory, because the 30-day survival after lethal whole body irradiation clearly indicates the capacity of the pharmacological agent in test to modulate the recovery and regeneration of the gastrointestinal epithelium and the hemopoietic progenitor cells in the bone marrow, the two most radiosensitive organs that are essential for sustenance of the life [131]. The most reliable procedures involve determination of a dose reduction factor (DRF). In animal studies, DRFs are typically determined by irradiating mice with or without administering radioprotective agent at a range of radiation doses and then comparing the endpoint of interest. For example, the DRF for 30-day survival (LD50/30 drug-treated divided by LD50/30 vehicle-treated) quantifies protection of the hemopoietic system [132,133]. With sufficient loss of hemopoietic stem cells, death follows due to infection, hemorrhage, and anemia. The GI syndrome in mice can be assessed by determining survival up to ten days (measure of GI death) after exposure to comparatively high doses of whole-body radiation, whereas hemopoeitic syndrome can be assessed by monitoring the survival of irradiated animals up to 30 days post-irradiation [134,135]. The intestinal crypt cell assay or functional changes also serve as indicators of GI damage [136]. The most informative and useful preclinical studies relate protective effects to the drug’s toxicity in the same animal model.

The efficacy of radioprotectors in clinical practice requires different end points. Among other endpoints amenable to the determination of beneficial effects of radioprotectors, the most readily evaluable is protection against mucositis and xerostomia resulting from head and neck radiotherapy and various side effects when the GI tract is in the radiation field [137].
2.6.4 Plants and Herbs as Radiation Protectors:

Several medicinal plants have been screened for their antigenotoxic activity. An intravenous infusion of an ethanol extract of *Gingko biloba* leaves, at a dose of 100 mg/person was found to be very effective in patients with vasogenic edema observed after irradiation of the brain [138]. It has been reported to be protective against the clastogenic factors from plasma of human subjects exposed to irradiation[139] Treatment of recovery workers from the Chernobyl accident site was found to be effective when an oral dose of 40 mg/day of *Gingko biloba* was given 3 times daily for 2 months. [140].

Aqueous extract of *Centella asiatica* reduced the adverse effect of low dose irradiation in Sprague Dawley rats by inhibiting radiation-induced body weight loss and conditioned taste aversion [141]. Similarly, it has been found to protect against the radiation-induced weight loss in mice exposed to 8 Gy γ-radiations [142].

Oral administration of a *Hippophae rhamnoides* fruit juice concentrate to rats before or after irradiation increased life span, restored the 11-oxytocorticosteroid level in the blood and weight of isolated adrenals, and also normalized their basal activity and response to (ACTH) (corticotropin) under in vitro conditions [143]. Hydroalcoholic extract of berries of *H. rhamnoides* also protected mice against γ-radiation-induced mortality, decline in endogenous colony forming unit (CFU), micronuclei formation and various other hematological parameters [144,145,146].

The radioprotective property of *Osimum sanctum* was first reported by Jagetia *et al*[147] against the radiation-induced mortality, thereafter studies by Uma Devi and Ganasoundari [148]established its radioprotective efficacy by evaluating mouse survival, spleen colony assay, and chromosome aberrations in mouse bone marrow cells. Apart from these osmium has been reported to
protect against radiation-induced lipid peroxidation and reduction in glutathione concentration [148,149].

The radioprotective efficacy of ginseng (*Panax ginseng*) has been reported by several workers [150,151,152,153, and 154]. Ginseng treatment caused recovery of thrombocyte and erythrocyte counts in blood after irradiation [155]. The whole extract of ginseng and the relative protective effects of various fractions (carbohydrate, protein and saponins) have been evaluated. The results showed that the water-soluble whole extract of ginseng provided best protection against radiation induced damage in C3H mice, whereas isolated protein and carbohydrate fractions were less effective, the saponin fraction was ineffective [156]. Similar results were obtained by Kim and coworkers [151], who found that whole ginseng extract and its fractions increased endogenous spleen colony formation in irradiated mice and also reduced apoptosis in jejunal crypt cells [151]. The radioprotective effect of ginseng root extract on testicular enzymes (acid and alkaline phosphatases and lipid peroxidation) has also been reported [152].

*Podophyllum hexandrum* has been reported to protect against radiation-induced mortality, gastrointestinal damage and embryonic nervous system of developing mice [146,157, 158]. It has also been reported to protect against radiation-induced decline in glutathione-S-transferase, superoxide dismutase in the liver and intestine of irradiated mice [159].

Oral administration of an aqueous extract of guduchi, *Tinospora cordifolia* has been reported to increase the survival of mice exposed to radiation [160]. Treatment of mice with hydroalcoholic extract of *Tinospora cordifolia* has been found to protect against the radiation-induced micronuclei formation and oxidative stress and decline in the mouse survival, spleen CFU and hematological parameters [161].
The fruit pulp of Amala, *Emblica officinalis* has been reported to increase the survival and inhibit radiation-induced weight loss in mice [162]. *Phyllanthus amarus* has been reported to protect against the radiation-induced decline in white blood cells (WBC), superoxide dismutase, catalase, glutathione-S-transferase, glutathione peroxidase, and glutathione reductase [163].

Daily oral administration of 800 mg/kg body weight of Rajgira, *Amaranthus paniculatus* leaf extract for 15 consecutive days before whole body exposure to γ-radiation protected mice against the radiation-induced lethality with a dose reduction factor of 1.36. It increased endogenous spleen colony forming units and spleen weight without any side effects or toxicity. Rajgara extract also arrested radiation-induced lipid peroxidation and the decline in reduced glutathione in the liver and blood of mice [164].

The ethanolic extract of *Piper longum* (pippali) fruits was found to protect mice against the radiation-induced decline in WBC, bone marrow cells α-esterase positive cells and GSH. Pippali extract also reduced the elevated levels of glutathione pyruvate transaminase (GPT), alkaline phosphatase (ALP), lipid peroxidation (LPO) in liver and serum of irradiated animals [165].

Several plant and herbal products form the supplements of daily human diet. The potential of dietary ingredients for radioprotection has remained unexplored area until now. The dietary supplements, if found radioprotective may be of crucial importance, as they are in daily human use, nontoxic and have wide acceptability. Jamun, *Syzigium cumini* Linn. Skeels also known as *Eugenia cumini* (family Myrtaceae), and has been reported to possess several medicinal properties in the folklore system of medicine [166]. The micronucleus study of radioprotective effect of dichloromethane and methanol (1:1) extract of jamun (SCE) in human peripheral blood lymphocytes (HPBLs)
ascertained its radioprotective potential, where 12.5 µg/ml SCE was found to reduce the micronuclei up to a maximum extent. In vivo evaluation further established its radioprotective activity where it was found to reduce radiation-induced sickness, gastrointestinal and bone marrow deaths [131,167]. Not only leaf but the hydroalcoholic extract of jamun seeds (JSE) also exhibited a greatest protective effect at 80 mg/kg JSE. The JSE was more effective when administered through the intraperitoneal route at equimolar doses than the oral. The JSE treatment protected mice against the gastrointestinal as well as bone marrow deaths with a DRF of 1.24 [168].

Pudina or Mint (*Mentha arvensis* Linn., Family Lamiaceae), a plant, native of Japan, is used as a food seasoner, household remedy, and for industrial purposes. Treatment of mice with 10 mg/kg b.wt. of chloroform extract of mint (*Mentha arvensis* Linn) protected against the radiation-induced sickness, gastrointestinal and bone marrow deaths with a DRF of 1.2. Further it was non-toxic up to a dose of 1000 mg/kg b. wt., the highest drug dose that could be tested for acute toxicity [169]. Pre-treatment of mice with leaf extract of another species of pudina, i.e. *Mentha piperita* has been reported to protect mice against the radiation-induced decline in hematological constituents, serum phosphatase, endogenous spleen colonies formation, spleen weight, goblet cells or villus section and chromosomal damage [170,171,172].

The rhizome of *Zingiber officinale*, commonly known as ginger, is consumed daily worldwide as a spice and flavoring agent. The rhizome of ginger has been reported to possess diverse medicinal properties in the traditional Indian system of medicine, the Ayurveda, and it is widely used in several medicinal preparations [166]. Administration of 10 mg/kg (i.p) or 250 mg/kg (orally) hydroalcoholic extract once daily, consecutively for 5 days was found to protect mice against the radiation-sickness, gastrointestinal as well as bone marrow deaths with a DRF of 1.15. Ginger has been reported to increase
glutathione, reduce lipid peroxidation in vivo and scavenging of various free radicals in vitro [173,174].

*Ageratum conyzoides*, (family: *Asteraceae*) is commonly known as Billy Goat Weed. It has been used in various parts of Africa, Asia and South America for curing various diseases. The study of various doses of alcoholic extract of *Ageratum conyzoides*, *Linn.* revealed that the best protective dose was 75 mg/kg and it reduced radiation-induced, sickness gastrointestinal as well as bone marrow deaths. A DRF was found to be 1.3. The radioprotective effect was due to scavenging of DPPH (1,1-diphenyl-2-picrylhydrazyl), free radical [175].

*Aegle marmelos*Correa, commonly known as bael, is a spinous tree belonging to family *Rutaceae*. It is grown throughout the sub-continents as well as Bangladesh, Burma and Srilanka [176]. The hydroalcoholic extract of *Aegle marmelos* (AME) protected cultured HPBLs against the radiation-induced micronuclei at a concentration of 5 µg/ml. It was also reported to scavenge ·OH, O2−, DPPH, ABTS+ and NO (nitric oxide) radicals *in vitro* in a concentration dependent manner [135]. The radioprotective efficacy of 15 or 250 mg/kg AME was further confirmed in animal studies where its intraperitoneal as well as oral administration has been found to protect mice against the radiation-induced sickness, gastrointestinal and bone marrow deaths and mortality giving a DRF of 1.2. It also protected mice against the radiation-induced lipid peroxidation and elevated GSH concentration in the liver, kidney, stomach and intestine at 31 days post-irradiation. Oral administration also protected mice against the gamma radiation-induced decline in erythrocytes, leukocytes, lymphocytes and clonogenicity of hemopoietic progenitor cells assessed by exogenous spleen colony forming assay. Pretreatment of mice with AME elevated the villus height and the crypt number accompanied by a decline in goblet and dead cell number [177,178]. Not only leaf but also the hydroalcoholic extract of Aegle marmelos fruit
administered intraperitoneally at a dose of 20 mg/kg once daily, consecutively for five days found to protect mice against the radiation-induced sickness, gastrointestinal as well as bone marrow deaths with a DRF of 1.1 [179].

The ethyl acetate fraction of *Aphanamixis polystachya* at a dose of 7.5 mg/kg b.wt. before exposure to 1–5 Gy of whole body gamma-radiation significantly reduced the frequencies of aberrant cells and chromosomal aberrations like acentric fragments, chromatid and chromosome breaks, centric rings, dicentrics, exchanges and total aberrations at all post-irradiation scoring times. It also showed a concentration dependent scavenging of hydroxyl, superoxide, 2,2'-diphenyl-1-picryl hydrazyl (DPPH) radicals and the 2,2-azino-bis-3-ethyl benzothiazoline-6-sulphonic acid (ABTS) cation radicals in vitro. EAP treatment also reduced lipid peroxidation in bone marrow cells in a concentration dependent manner [180].

### 2.6.5 Mechanism of Action

The ionizing radiations induce reactive oxygen species in the form of ·OH, ·H, singlet oxygen and peroxyl radicals that follows a cascade of events leading to DNA damage such as single- or double-strand breaks (DSB), base damage, and DNA-DNA or DNA-protein cross-links. These lesions cluster as complex local multiply damaged sites. The DNA-double-strand breaks are considered the most lethal events following ionizing radiation and have been found to be the main target of cell damage and cell death followed by radiation exposure [181]. The putative mechanisms of radiation protection by medicinal plant and herbal radiation protectors are studied. The antigenotoxic activity of medicinal plant and herbs may be mediated through several mechanisms, since they contain complex mixtures of many phytochemicals.

Majority of the medicinal plants and herbs contain polyphenols, which scavenging for radiation-induced free radicals and elevation of cellular antioxidants by medicinal plants and herbs in irradiated systems could be
leading mechanisms for radiation protection. The polyphenols that are present in the plants and herbs may upregulate the mRNAs of antioxidant enzymes such as catalase, glutathione transferase, glutathione peroxidase, superoxide dismutase and thus may counteract the oxidative stress-induced by ionizing radiations.

2.7 ROLE OF INDIAN MEDICINAL PLANTS IN HEALTH CARE

A variety of medicinal plants that were traditionally used for thousands of years in India are found to be present in a group of herbal preparations of the Indian traditional health care system. This traditional health care system is known as Ayurveda and is also named as Rasayana. These Indian medicinal plants have been proposed for their interesting antioxidant activities, wound healing activities and other medicinal properties with no serious side effects. Among the Indian medicinal plants that have been used for the treatment of wounds and skin disorders, the following six Indian medicinal plants have been thoroughly investigated in the present study. These medicinal plants are studied for their historical, etymological, morphological, phytochemical and pharmacological aspects. The plants described contain antioxidant principles, which can explain and justify their use in traditional medicine in the past as well as in the present.

2.7.1 Scientific Evaluation of the Indian Medicinal Plants

Many interesting studies have been performed to identify the antioxidant compounds with an excellent pharmacological activity with a limited toxicity. In the present context, ethno pharmacology represents the most important way that is possible for finding interesting and therapeutically useful molecules. The phytochemical analysis of the medicinal plants used in rasayana has revealed to possess a large number of compounds including tannic acid, flavonoids, tocopherol, curcumin, ascorbate,
carotenoids, polyphenols, etc. which have been identified to have potent antioxidant activities [21,182,22]. The herbal preparations that were used in Indian traditional medicine may have antioxidant activities and wound healing activities arising from their content of medicinal plants with antioxidant principles, which act probably in a synergistic way. This hypothesis along with excellent pharmacological activity and lack of toxicity [23] can be shown to have important basis to understand their medicinal properties and therapeutic uses in the past as well as now.

There is an enormous increased quest to obtain natural antioxidants from medicinal plants with broad-spectrum actions. The majority of the rich diversity of Indian medicinal plants is yet to be scientifically evaluated for such medicinal properties and therapeutic actions. Furthermore, the relationship between phenolic content and its antioxidant activity is largely not yet examined properly in Indian medicinal plants. Among the enormous variety of Indian medicinal plants that are commonly used in the Indian system of medicine, the above mentioned six Indian medicinal plants were selected for the present study. These medicinal plants were widely used for the treatment of wounds and injuries and other disorders of the skin from time immemorial which needs scientific evidences.

2.8 REVIEW OF SIX INDIAN MEDICINAL PLANTS

2.8.1 Indigofera aspalathoides VAHL.

*Indigofera aspalathoides* (Leguminosae) is commonly known as ‘Shivanarvembu’ in Tamil. In the traditional medicinal system, the leaves, flowers and tendershoot are said to be cooling and demulcent. They are used in the form of decoction for the treatment of leprosy and cancer [183]. The leaves are also applied to abscesses. The whole plant is used in oedematous tumors and the ashes are used in preparations of medicine for treating dandruff [184]. The methanolextract of *Indigofera aspalathoides* also possess hepatoprotective
activity [185]. The antitumor activity of the ethanol extract of *Indigofera aspalathoides* was established [186].

*Indigofera aspalathoides* which belongs to *Papilionceae* family is a low under shrub widely distributed in South India and Sri Lanka. Siddhaphysician traditionally used the leaves and flowers of this plant to treat elephantiasis, skin disorders like psoriasis, leprosy and cancer [20]. Studies with the stem extract clearly indicate that it has antitumor, antiviral and antibacterial effect [187, 186]. The possible antioxidant potential of the ethanolic and chloroform fractions obtained from the leaves of *Indigofera aspalathoides* have been explored using *in vitro* experiments [187].

*Indigofera aspalathoides* has demonstrated a remarkable chemopreventive effect in chemical-induced carcinogenesis in mouse. The potential chemopreventive action of *Indigofera aspalathoides* may be due to its antioxidant and detoxifying properties [188]

2.8.2 *Myristica andamanica* HOOK.F.

*Myristica andamanica* is used by Jarawas tribes of the Andaman and Nicobar islands for the treatment of skin diseases, to stop bleeding and other general sickness. *Myristica andamanica* is an aromatic, carminative, hallucinogenic, stimulant that is considered effective in digestive disorders, dehydration and skin disorders. Detailed investigations and studies on the phytochemistry and biological activities of *Myristica andamanica* have not been reported.

2.8.3 *Adhatoda vasica* NEES.

*Adhatoda vasica* Nees (family *Acanthaceae*) is commonly known as Vasaka or Arusha. The Vasakaplant is a perennial, evergreen and highly branched medicinal plant with unpleasant smell and bitter taste. The plant lives for multiple seasons and retains its leaves throughout the year. It is a shrub of 1.0 m to 2.5 m in height, with opposite ascending branches. The drug
formulation contains stem, leaf, flower, fruit and seeds [189,190]. The plant grows throughout India. It is commonly found in sub Himalayan track up to an altitude of 1000 meters above sea level and in Maharashtra especially in Konkan region. Besides India, it is found in Myanmar, Sri Lanka, Burma and Malaysia [191].

Vasaka is a bitter vasicine and vasicinone which are present in all parts of the plant. The leaves of *Adhatoda vasica* contain several alkaloids (vasicinone, vasicinol, adhatodine, adhatonine, adhavasinone, anisotine and peganine), betaine, steroid carbohydrate and alkanes. In the flowers of *Adhatoda vasica* triterpines (aamirine), flavonoids (apigenin, astragalin, kaempferol, quercetin, vitexin) have been found [191,189,192]. *Adhatoda vasica* is a good source of vitamin C and has medicinal uses, mainly as antispasmodic, antipyretic, anti-inflammatory, anti-bleeding, branchodilator, anti-diabetic, disinfectant, anti-jaundice, oxytocic and expectorant [191]. Most of these attributes fall mainly into respiratory therapy category for cold, asthma, bronchitis and tuberculosis. Antioxidant activity of plants might be due to their phenolic compounds [193].

Phytochemical studies showed that the extract of *Adhatoda vasica* contain high amount of phenolic compounds and exhibited antioxidant activity. The high free radical scavenging property of *Adhatoda vasica* may be due to hydroxyl groups existing in the phenolic compounds. The free radicals are often generated as byproducts of biological reactions or from exogenous factors. The involvement of free radicals in the pathogenesis of a large number of diseases has been studied [194,190].

### 2.8.4 *Azadirachta indica* A. JUSS.

*Neem* (*Azadirachta indica* A. Juss. *Meliaceae*) is an attractive, evergreen medicinal tree that can grow up to a height of 20 m tall. The huge branches form rounded crowns up to 10 m in diameter. The short and usually
straight trunk has a moderately thick and strongly furrowed bark that has garliclike odour and a bitter, astringent taste. It’s leaves are imparipinate, 20 to 38 cm long and crowded near the branch end, oblique, lanceolate and deeply and sharply serrate. *Azadirachta indica* is rarely leafless and is usually in full foliage even during months of prolonged drought. It’s small, white bisexual and staminate which are functionally male, flowers are borne in axillary clusters. They have a honeylike scent and attract many bees which act as pollinators. The fruit is a smooth, ellipsoidal drupe, 1 to 2 cm long that is yellow when mature and comprises a sweet pulp enclosing a seed. The seed is composed of a shell and a kernel sometimes two or three kernels, the latter having a high oil content. Neem will begin bearing fruit after 3 to 5 years, becomes fully productive in 10 years and can produce up to 50 kg of fruits annually [195,196,197,198,199].

Floral initiation occurs in *Azadirachta indica* during a short period of leaf shedding [198], after which flowering will last for about five weeks, with the flowers opening in succession. *Azadirachta indica* will normally begin flowering and producing seed after 3 to 5 years of age, but flowering and fruiting can be extremely variable within the species [200,201,202,203]. For example, Gogate and Gujar [202] found flowering and fruiting in a 50-year-old plantation of neem in Maharashtra, India to be highly variable. Out of 331 trees understudy, a total of 292 flowered and fruited in the 1992 season, and fruit production varied from 7.5 kg to 17.3 kg dry weight per tree. Studies on the fructal phenology of *Azadirachta indica* in a threearold provenance trial at Jodhpur, India revealed that 25.52% of the trees in the trial were fruiting in the third year, although this varied between provenances from 0 to 65% [203]. The majority of the trees were fruiting synchronously with a small percentage of about 3.72% flowering while the other trees were fruiting. The timing of flowering and fruiting varies from site to site, but in India, neem flowers in April and the fruits are ready for harvesting in July. Late flowering genotypes
have, however, been identified from Tirupur, Tamil Nadu, India [204]. The genotype flowered and fruited at the normal time and from September to December. Seed germination was reduced in the lateflowering type, and a higher proportion of abnormal seedlings were produced. These abnormalities may be due to an increased level of selfing in the lateflowering type.

2.8.4.1 Use of *Azadirachta indica* in health and medicine

*Azadirachta indica* has been used for thousands and thousands of years to cure diseases in humans. A survey conducted in Nigeria found that the predominant use of *Azadirachta indica* was for medicinal purposes [22]. Similarly, a recent study by GTZ [205] showed that the knowledge of a population about the use of *Azadirachta indica* for medicine ranged from 63% in Caribbean/Latin America to 86% in Africa and 93% in Asia. The same survey indicated that the leaves of *Azadirachta indica* were the most commonly used part of the tree for medicine. A common use of *Azadirachta indica* is to control fevers, in particular malaria, where a tea made with the leaves is drunk.

*Azadirachta indica* is reported to have anti-fertility, anti-bacterial, anti-fungal, anti-inflammatory and anti-diabetic effects [206]. In recent years, extensive research into the potential use of *Azadirachta indica* as a medicine has increased. A polyherbal formulation containing *Azadirachta indica*, Praneem, has been developed in India for contraceptive use [207]. The polyherbal formulation has a potent spermicidal effect and inhibits the growth of *Candida* spp., *Chlamydia trachomatis* and urinary *E. coli*. Clinical trials have been conducted in India, Brazil, Egypt and the Dominican Republic, although the product is not yet licensed for use. There are also claims that neem has anti-veridical properties and can be effective against diseases such as HIV-1. Anti-cancer properties have also been demonstrated *in vitro* [208].
The commercial market for neem-based health and beauty products is better developed and accepted than that for neem-based medicines. In India, Khadi Village Industries has been responsible for the development of village cottage industries that include soapmaking. Soap products made with neem oil currently represents main use of neem oil in India. Manufacturing neem oil soaps may be an appropriate income generating activity for women. A community group in northern Ghana recently visited a women's group in Burkina Faso where they were exposed to several income generating activities, but chose to adopt neem oil and soap manufacture [209].

Many researchers have commented on the potential use of *Azadirachta indica* as an ingredient for pharmaceutical and household products. For example, *Azadirachta indica* is used to kill or repel dust mites, cockroaches, ants, fleas, ticks and moths which cause human allergies, diseases and destroy textiles. The consumer appears to be prepared to pay high prices for such products because of the adverse effect of some existing products and the biodegradable nature of *Azadirachta indica*.

As research leads into compounds derived from *Azadirachta indica* continues, it is likely that consolidation of data will occur and it is quite possible that *Azadirachta indica* may be identified as a source of cost-effective compounds capable for use in Western medicine or health products. However, the medicinal value that people attribute to *Azadirachta indica* all over the world and the fact that *Azadirachta indica* is used repeatedly from generation to generation, suggests that the tree has an important role to play in local medicine and is of great benefit to the disenfranchised and poor who often cannot afford the cost of Western medicines.

*Azadirachta indica* is a unique source of various types of phytochemical compounds having diverse chemical structures. Very little research work has been done on the biological activity and plausible medicinal applications
of these phytochemical compounds and hence extensive investigations on the mechanism of action are needed to exploit their therapeutic utility to combat diseases. A drug development programme should be undertaken to develop modern drugs with the compounds isolated from *Azadirachta indica*. Although crude extracts from various parts of neem have medicinal applications from time immemorial, modern drugs can be developed after extensive investigations are done on its bioactivity, mechanism of action, pharmacotherapeutics and toxicity and after standardization and clinical trials. As the global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use, development of modern drugs from *Azadirachta indica* should be emphasized for the control of various diseases. In fact, this is the apt period to make good use of old and valuable knowledge on *Azadirachta indica* through modern approaches of drug development. For the last few years, there has been an increasing trend and awareness in research on *Azadirachta indica*. Quite a significant amount of research has already been carried out during the past few decades in exploring the phytochemistry of different parts of neem. Several therapeutically and industrially useful preparations and compounds have also been marketed which generates enough encouragement among the scientists in exploring more information about this age-old medicinal plant. An extensive research and development work should be undertaken on *Azadirachta indica* and its products for their better economic and therapeutic utilization.

More than 135 phytochemical compounds have been isolated from different parts of *Azadirachta indica*. These phytochemical compounds are classified into two major groups, namely, isoprenoids and non-isoprenoids. The isoprenoids include diterpenoids and triterpenoids containing protomeliacins, liminoids, azadirone and its derivatives, genudin and its derivatives, vilarin type of compounds and csecemeliacinssuch as nimbin, salannin and azadirachtin. The first compound to be studied was nimbin. The
non-isoprenoids include proteins (aminoacids) and carbohydrates (polysaccharides), sulphurous compounds, polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds, phenolic acidsetc. [210,211,212,213,214,215].

2.8.4.2 Medicinal uses

Since time immemorial, Indians are aware of the medicinal properties of *Azadirachta indica*. *Azadirachta indica* has beenextensively used in Ayurveda, Unani and Homeopathic medicine. Traditionally, many disorders like inflammation, infections, fever, skin diseases, dental disorders and others have been treated with different parts of neem tree such as leaves, flowers, seeds, fruits, roots and bark. *Azadirachta indica* leaves exhibit a wide range of pharmacologicalactivities *viz.*., anti-inflammatory, anti-hyperglycaemic, anti-ulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic anticarcinogenic and immunomodulatory [216].

Anticarcinogenic activity of neem leaf extract was observed in murine system [217]. Injection of neem leaf preparation to tumor in mice reduced tumor growth, exhibiting anticarcinogenic activity [218]. Induction of apoptosis in rat oocytes was seen when treated with neem leaf extract [219]. Buccal pouch carcinogenesis in hamsters was inhibited by ethanolic leaf extract of neem [220]. The ethanolic leaf extract of neem also caused cell death of prostate cancer cells (PC-3) by inducing apoptosis [221]. Good antioxidant activity was observed with neem leaf aqueous extract; flower and stem bark ethanolic extracts [222]. Administration of aqueous extract of neem along with DOCA salt prevented the development of hypertension in rats [223].

Neem leaf extracts are antimutagenic. The ethanolic extract of neem leaves exhibited strong antimutagenic activity in *Channa punctatus*, a fresh water fish model [224]. Aqueous extract of neem root and leaves reduced blood sugar level in rats exhibiting antidiabetic activity [225]. The bark
extract completely healed the duodenal ulcers when administered at the dose of 30-60 mg twice daily for 10 weeks. Neem bark extract had potential of controlling gastric hypersecretion, and gastroesophageal and gastroduodenal ulcers [226].

Acetone-water neem leaf extract showed antiretroviral activity through inhibition of cytoadhesion. The extract increased haemoglobin concentration, mean CD4+ cell count and erythrocyte sedimentation rate in HIV/AIDS patients [227]. Enhancement of antibody production and cellular mediated response by neem components helps in the treatment of AIDS.

Neem leaf and seed extracts exhibited antidermatophytic activity against dermatophytes viz., Trichophyton ruberum, Mentagrophytes, Trichophyton violaceum, Microsporum nanum and Epidermophyton floccosum under in vitro conditions [228]. Neem seed oil showed bactericidal activity against 14 strains of pathogenic bacteria [229]. Crude aqueous and solvent extracts of neem were tried against 20 strains of pathogenic bacteria where in crude extract produced better results [230]. The contraceptive property of neem oil has been reported [231,232]

2.8.5 Saraca asoca ROXB.

Saraca asoca is widely distributed in evergreen forests of India up to an elevation of about 750 meters and is found throughout India especially in Himalaya, Kerala, Bengal and the whole south regions. In Himalaya, it is found at Khasi, Garo and Lussi hills. In Kerala, it is found in Patagiri, Kaikatty and Pothundi of Palakkad district, Thrissur, Kollam and Kannaur districts [233].

Saraca asoca is a small evergreen tree of 7 to 10 m height. Leaves are parpinnate of 15 to 20 cm long and the leaflets are 6 to 12, oblong and rigidly sub-coriaceous. Leaves are narrowly lanceolate, cork like at the base and with a short pestistipules are intra-petiolar and completely united. The bark is dark
brown or grey or almost black with warty surface. The stem bark are rough and uneven due to the presence of rounded or projecting lenticles and the bark channeled, smooth with circular lenticles and traversely ridged, sometimes cracked. Fracture splinting exposing striated surface, a thin whitish and continuous layer is seen beneath the cork leaver. Flowers are fragrant. The flowers are polygamous apetalous, yellowish orange turning to scarlet, in short laterally placed corymbose, axillary panicles, bracts small, deciduous, calyx petaloid. The seeds are 4 to 8, ellipsoid oblong and compressed [234,235].

2.8.5.1 Phytochemistry

The phytochemical studies of the bark have shown the presence of (-) epicatechin, procyanidin 2,11'-deoxyprocyanidin B, (+) catechin, (24, £)-24-methyl-cholest-5-en-3β-ol (22 E, 21£)-24-ethylcholesta-5,22 dien-33-ol,(24 £)-24-ethylcholesta-5-en-3-p-ol, leucopelargonidin-3-O-p-D-glucoside, leucocyanidin and leucopelargonidin. The flowers were observed to contain oleic acid, linoleic acid, palmitic acid and stearic acid, P-sitosterol, quercetin, kaempferol-3-0-P-D- glucoside, quercetin-3-0-P-D-glucoside, apigenin-7-0-p-D-glucoside, pelargonidin-3,5-digloside, cyanidin-3,5-digloside, palmitic, stearic, linolenic, linoleic, p and y sitosterols, leucocyanidin and gallic acid. Seed and pod contains oleic, linoleic, palmitic and stearic acids, catechol, (-) epicatechol and leucocyanidin [234,235]. Five lignan glycosides, lyoniside, nudiposide, 5-methoxy-9-β-xylopyranosyl(−)-isolariciresinol, icariside E3, and schizandriside, and three flavonoids, (−)-epicatechin, epiafzelechin-(4β→8)-epicatechin and procyanidin B2, together with β-sitosterol glucoside, were isolated from dried bark.

2.8.5.2 Antimicrobial Activity

*Saraca asoca* extract prepared from ethanol and water in the ratio of 1:1 was subjected to antibacterial activity on agar plate with different plant pathogens such as *Bacillus subtilis, Escherichia coli, Salmonella typhosaand...
*Staphylococcus aureus.* *Saraca asoca* flower buds evaluated against antibacterial activity of methanolic extract on agar plate against *Salmonella viballerup, Shigella boydii, Escherichia coli, Vibrocholera, Shigella flexneri* and *Shigella dysenteriae* showed positive activity. *Saraca asoca* leaves extract prepared from 95% ethanol and water was investigated against antibacterial activity on agar plate against *Escherichia coli* and *Staphylococcus aureus.* Activity against *Escherichia coli* was found positive whereas activity against *Staphylococcus aureus* gave negative result. The methanolic extracts of *Saraca asoca* was assayed against *Alternaria cajani, Helminthosporium sp., Bipolaris sp., Curvularia lunata* and *Fusarium sp.* at different concentrations of 1000, 2000, 3000, 4000 and 5000 µg/ml. The extract exhibited good inhibitory activity against *A. cajani,* while it was to be effective at lower concentrations against other fungi also. Four different extracts of *Saraca asoca* bark was tested for antibacterial activity against *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus, Klebsiella aerogenes, Shigella boydis, Proteus vulgaris.* Different extracts of *Saraca asoca* bark were screened against the enteric pathogen isolates, namely, *Escherichia coli, Shigella sonnei* and *Salmonella enteritis.* All the extracts other than aqueous extract showed antifungal activity with the methanol extract having the highest percentage of activity. Methanol and water extracts of *Saraca asoca* leaves exhibited good activity against *Bacillus subtilis, Pseudomonas aeruginosa* and *Salmonella typhymurium.* Both extracts showed marked activity against *Alternaria alternate, Colletotrichum gloeosporioides* and *Drechlera specifera.* The crude extracts of leaves, flowers, and bark of *Saraca asoca* were screened for larvicidal activity for 24 to 48 hours at an initial concentration of 1000 ppm against early IV instar larvae of the vector mosquitoes namely, *C. quinquefasciatus, A. aegypti,* and *A. stephensi.* The petroleum ether extract of *Saraca asoca* leaves and chloroform extract of *Saraca asoca* bark exhibited more than 50% larval mortality against *C. quinquefasciatus* larvae at an exposure period of 48 hours.
2.8.5.3 Anticancer Activity

The anticancer principle compound from *Saraca asoca* flowers indicated 50 percent cytotoxicity *in vitro* in Dalton's lymphoma ascites and Sarcoma-180 tumour cells at a concentration of 38 µg and 54 µg respectively and with no activity against normal lymphocytes but preferential activity for lymphocytes derived from leukemia patients.

2.8.5.4 Antimenorrhagic Activity

*Saraca asoca* dried bark has been used for treatment of menorrhagia in India. In India, dried bark as well as flower of *Saraca asoca* is given as a tonic to ladies to treat uterine disorders. *Saraca asoca* stem bark is also used to treat all disorders associated with the menstrual cycle. In Sri Lanka, *Saraca asoca* bark has been used for treating menstrual disorders and menorrhagia in Sri Lanka. In India, *Saraca asoca* bark has been used as a uterine sedative and hot water extracts administered to human adult females stimulates the uterus similar to ergot, but without producing tonic contraction. *Saraca asoca* has been employed in treating menorrhagia, as an emmenagogue, uterine sedative and uterine infections as well as used in several preparations related to gynecological problems. In Pakistan, *Saraca asoca* bark has been employed for uterine infections and menorrhagia. In India, *Saraca asoca* dried bark has been used as an astringent in menorrhagia, to stop excessive uterine bleeding and also as a refrigerent, demulcent, uterinedisorders and regular menstrual pain in abdomen and used for uterine problems. Aqueous extract of the bark has been reported to contain active principles, one stimulating and the other relaxing the plain muscle of the ileum of the guinea pig. The drug has been reported to stimulate the uterus, making the contraction more frequent and prolonged. The crystalline glycoside substance is also reported to stimulate uterine contraction.
2.8.5.5 Antioxytocin Activity

Oxytocin activity of *Saraca asoca* was seen in isolated uterine preparations of rat and human. Estrogenprimed gravid uterus was more sensitive to the action of the alcoholic extract. Pentoliniumbitartrate completely blocked the oxytocin action. Seed extract was found effective against dermatophytic fungi. *In vitro* studies on rat uterus preparation using extracts of *Saraca asoca* did not show oxytocin activity. *Saraca asoca* has been studied twice previously with negative results and once with positive results.

*Saraca asoca* is regarded as a universal panacea in the Ayurvedic medicine. It is one of the universal medicinal plant having medicinal activities. This versatile plant is an important source of various types of phytochemical compounds. In the present scenario many medicinal plants are used to treat various diseases. But *Saraca asoca* is an ancient and reliable source of medicine and has many pharmacological activities like anticancer activity, antimenorrhagic activity, anti oxytocic activity, antimicrobial activity and have extend uses in Ayurveda, Unani and Homeopathy. It has many medicinal and therapeutic properties against skin infections, CNS function, genitor urinary functions. As the global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use, and development of modern drugs from *Saraca asoca* should be emphasized for the control of various diseases.

2.8.6 *Aeglemarmelos* Linn.

*Aegle marmelos* is one of the most important and widely used medicinal plants of India, Burma and Srilanka[236]. It is found as a wild plant in the central and south India and mostly cultivated in north India. *Aegle marmelos* is a subtropical species. It grows up to an altitude of 1200 m in Punjab where the temperature increases to 48.89°C in summer and decreases to -6.67°C in winter and prolonged droughts occur[237]. *Aegle marmelos* is a
slow growing, medium sized tree that grows up to 12 to 15 m tall with short trunk, thick, soft, flaking bark and spreading sometimes spiny branches in which the lower ones are found to be drooping. Young suckers bear many stiff and straight spines. A clear gummy sap resembling gum Arabic exudes from the wounded branches and hangs down in long strands which gradually solidify. It is sweet at first taste and then irritates down the throat.

*Aegle marmelos* has enormous indigenous therapeutic uses against various diseases and many bioactive compounds have been isolated from this plant also [238]. This plant is used in traditional treatments such as intermittent fever, intestinal ailments, fertility control and treatment after childbirth and fish poison [239]. The effectiveness of *Aegle marmelos* fruit in treating diarrhoea and dysentery has resulted in its entry into the British Pharmacopoeia [240]. Chopra has aptly stated that “No drug has been longer and better known nor more appreciated by the inhabitants of India than the *Bael* fruit”.

A survey showed that in 2001 to 2002 in the Himalayan region in the State of Uttaranchal, Indian Republic, Vaidas who are the Practitioners of Ayurveda used *Aegle marmelos* as an ingredient in the respective herbal formulations for boils, dysentery, ear aches and discharge from the ears, fever and cold. The ripe fruit, the unripe fruit, the roots, the leaves and the branches have all been used in traditional medicine. In Ayurveda, the ripe fruit has been used for chronic diarrhea and dysentery, as a tonic for the heart and brain, and as an adjuvant treatment for dysentery. Decoction prepared from the root has been used to treat melancholia, intermittent fevers and palpitation. The roots have been used as an ingredient of the Ayurvedic medicine, Dashmool. The leaves have been given as febrifuge and as a poultice for the treatment of eye disorders and ulcer. Administration of fresh leaves has been used for weakness of the heart, dropsy and beriberi.
2.8.6.1 The ethno medicinal uses of leaves of *Aegle marmelos*

Paste of leaves is applied to inflamed parts and is very effective in the form of poultice to unhealthy ulcers. Young leaves when eaten cause sterility and abortion. Juice of fresh leaves has alaxative action and also employed in treating asthma, ophthalmia and eye infections. Decoction of leaves is used as a febrifuge and expectorant. Medicated oil prepared from the leaves gives relief from recurrent cold and other respiratory infections. The juice extracted from leaves is mixed with equal quantity of sesame oil and heated thoroughly and a few seeds of black pepper and half a teaspoonful of black cumin are added to hot oil and is removed from the fire and stored for use when necessary. A teaspoon of this oil should be massaged onto the scalp before head bath. Its regular use builds resistance against cold and cough. Leaves are also used in abscess, backache, abdominal disorders, vomiting, cut and wounds, dropsy, beriberi, weakness of heart, cholera, diarrhea, cardiac tonic, blood sugar, injuries caused by animals, nervous disorders, hair tonic, acute bronchitis, child birth [241]. It is also used in veterinary medicine for wound healing, killing worms, fodder for sheep, goat and cattle, stimulation of respiration contraction of denervated nictitating membrane in anaesthetized cats [242].

2.8.6.2 Phytochemical constituents isolated from *Aegle marmelos*

Various phytochemical constituents like alkaloids, coumarins and steroids have been isolated and identified from different parts of tree. Coumarins, marmelosin, marmesin, imperatorin, marmin, alloimperatorin, methyl ether, xanthotoxol, scopoletin, scoparone, umbelliferone, psoralen and marmelide have been reported [243]. Marmenol, a 7-geranyloxy coumarin [7-(2,6-dihydroxy-7-methoxy-7-methyl-3-octaenoxy) Coumarins] has also been reported [244].
2.8.6.2.1 Alkaloids

Aeglin, aegelenine, dictamine, fragrine (C\textsubscript{13}H\textsubscript{11}O\textsubscript{3}N), Omethylhalfordinine,isopentenylhalfordinol [243]; N-2-[4-(3’, 3’-dimethylallyloxy) phenyl] ethyl cinnamide,N-2-hydroxy-2-[4-(3’, 3’-dimethylallyloxy) phenyl] ethylcinnamide, N-2-hydroxy-(4-hydroxyphenyl) ethylcinnamide [245]; O-(3, 3-dimethylallyl) halofordinol, N-2-ethoxy-2-(4-methoxy phenyl) ethyl cinnamide, N-2-methoxy-2-[4-(3’, 3’-dimethylallyloxy) phenyl] ethylcinnamide, N-2-methoxy-2-(4-methoxyphenyl)-ethylcinnamide [246] have been reported.

2.8.6.2.2 Polysaccharides

Galactose, arabinose, uronic acid and L-rhamanose areobtained on hydrolysis [247].

2.8.6.2.3 Seed oil

Seed oil is composed of palmiticacid, stearicacid, oleicacid, linoleicacid and linolenicacid [243].

2.8.6.2.4 Tannins

The maximum tannin content in \textit{Aegle marmelos} fruit was recorded inthe month of January. There is as much as 9% tannin inthe pulp of wild fruits and less in cultivated crops. Tannin isalso present in leaves as skimmianine and is namedas 4, 7, 8-trimethoxyfuro, quinoline.

2.8.6.2.5 Carotenoids

Carotenoids are responsible for imparting pale color to the fruit. Marmelosin, skimmianine and umbelliferone are thetherapeutically active principles of \textit{Aegle marmelos}. Minorconstituents like ascorbic acid, sitosterol, crude fibres,tannins, α-amyrin, carotenoids, and crude proteins arealso present. Roots of the tree have also been found tocontain psoralen, xanthotoxin scopoletin [243].Compounds such as praealtin D, trans-cinnamic acid, 4-
methoxy benzoic acid, betulunic acid and montanin have also been reported [248]. A large number of bioactive compounds have been isolated from various part of the *Aegle marmelos*.

2.8.6.3 Bioactivity

*Aegle marmelos* is an important medicinal plant of India. Leaves, fruits, stem and roots of *Aegle marmelos* have been used in ethno medicine to exploit its medicinal properties including astringent, antidiarrheal, antidysenteric, demulcent, antipyretic and anti-inflammatory activities [238]

2.8.6.4 Antioxidant activity

Antioxidant parameters like reduced glutathione, glutathione peroxidase, glutathione reductase, superoxide dismutase (SOD) and catalase have shown adoserelated increase in their level and activity and a decrease in lipid peroxidation following the treatment with *Aegle marmelos* leaf extract [249]. The fruit extract at a dose of 250 mg/kg body weight is more effective than glitenclamide at a dose of 300 µg/kg [250]. Leaf extract at a dose of 200 mg/kg is as effective as alpha tocopherol at a dose of 60 mg/kg in isoproterenal (ISO) treated rats [251]. The antioxidant phytochemical such as flavonoids, alkaloids, sterols, tannins, phlobotannins and flavonoid glycosides present in the leaf extract possess free radical scavenging activity [252]. Glutathione (GSH) is reduced in erythrocyte whereas plasma glutathione-S-transferase (GST) and malodialdehyde (MDA) are increased in male albino rats with diabetes. However, these alterations returned to normal level with *Aegle marmelos* leaf extract administration, suggesting antioxidant potential of *Aegle marmelos* leaves [253]. Eugenol and marmesinin may be responsible for such antioxidant activity because these compounds have independently shown their antioxidant activity against oxidativestress [254].
### 2.8.6.5 Antimicrobial activity

Various extracts of *Aegle marmelos* leaves, roots and fruits have been reported to be active against many bacterial strains. Leaf extracts have shown activity against *Escherichia coli* [255]. The essential oil obtained from the leaves of *Aegle marmelos* exhibited activity against *Aeromonas sp.*, *E. coli*, *Pseudomonas salanacearum* and *Xanthomonas vesicatoria* [256]. The ethanolic extract of the root has shown activity against *Vibrio cholerae*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *E. coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* [257]. The ethyl acetate extract of the plant has exhibited activity against *Vibrio cholerae*, *S. typhi*, *S. aureus*, *Pseudomonas putida* and *Bacillus anthracis* [258].

Methanol and aqueous extract of *Aegle marmelos* fruit have shown strong activity against multidrug resistant *S. typhimurium*. Methanolic extract is more potent than the aqueous extract. The minimum inhibitory concentration (MIC) value of the methanolic extract is observed to be around 256 μg/ml. The unsaponifiable matter of the seed has shown considerable in vitro activity against *E. coli*, *S. typhi*, *Salmonella paratyphi*, *Proteus vulgaris*, and *Streptococcus faecalis*, *V. cholerae*, *Klebsiella pneumoniae*, *P. aeruginosa* and *Neisseria gonorrhoeae* [259]. Both the oil and unsaponifiable matter of the seed have also been found to be active against *B. subtilis*, *E. coli*, *Klebsiella aerogenes*, *S. typhi*, *S. paratyphi*, *S. aureus*, *Erwinia carotovora*, *Pseudomonas solanacearum*, *Xanthomonas citri* and *Xanthamalvacearum* [259]. Thus it is evident that *Aegle marmelos* has antibacterial activity and the mechanism of action may be the blockage of protein synthesis either at the transcription or the translation level and the peptidoglycan synthesis at membrane level. The antibacterial activity of the leaf extract may be due to the presence of cuminaldehyde and eugenol because these compounds have already been observed for their activities against various bacterial strains [260].
The *in vitro* viral activity of various parts of *Aegle marmelos* has been evaluated for their efficacy against human Coxsvackie viruses B1-B6. The IC₅₀ value of leaves, stem and stem bark, fruit, root and root bark and pure compound marmelide are 1000, 500 to 1000, 250 to 500, and 62.5 μg/ml, respectively, whereas, the IC₅₀ of ribavirin, a standard antiviral agent, is 2000 μg/ml for the same viruses and at the same time period [261]. Marmelide is the most effective virucidal agent interfering with early events of its replication cycle[261]. It seems that *Aegle marmelos* has antiviral activities in the early stages of viral replication with minimum host cytotoxicity in contrast to modern virucidal chemotherapeutic agents like ribavirin, which usually act in the later stages of viral replication and have potent side effect[262].

### 2.8.6.6 Antigenotoxic activity

The radio protective effect of hydro alcoholic extract of *Aegle marmelos* leaves has been evaluated in cultured human peripheral blood lymphocytes (HPBLs). The irradiation of HPBLs with different doses of gamma radiation caused a dose-dependent increase in the frequency of lymphocytes bearing one, two and multiple micronuclei. Treatment of HPBLs with 5 μg/ml leaf extract significantly reduced the frequency of lymphocyte bearing one, two and multiple micronuclei when compared with their irradiated control. The mechanism of action of this type of radioprotective activity of the leaf extract may be due to the scavenging of radiation induced free radicals [135]. Radio protective activity of *Aegle marmelos* leaf extract has also been studied in male Swiss albino mice. The mice were administered with various intraperitonéalsingle dose of the extract of *Aegle marmelos*. The optimum radio protective dose of the extract has been observed to be five consecutive doses of 15 mg/kg body weight [177](Jagetia *et al.*, 2004). Irradiation caused a dose dependent decline in the level of glutathione and elevation in the lipid peroxidation. *Aegle marmelos* leaf extract arrested glutathione decline and lipid peroxidation significantly [263]. Hydro alcoholic extract of *Aegle marmelos*
fruit has also been studied for its radio protective effect in mice exposed to various doses of gamma irradiation. A dose of 20 mg/kg administered intraperitoneal for 5 consecutive days before irradiation of gamma rays has been found to afford maximum protection as is evident by the highest number of survivors after 30 days of postradiation [177]. Symptoms of sickness and mortality of the mice are due to irradiation resulting in a dose dependent elevation in lipid per oxidation in liver, kidney, stomach and intestine as well as depletion in GSH concentration. Treatment of the Aegle marmelos fruit extract before irradiation caused a significant decrease in the lipid per oxidation accompanied by a significant elevation in the GSH concentration in liver, kidney, stomach and intestine of mice [177].

Detailed information on the phytochemicals and various biological properties of the plant extracts might provide detailed evidence for the use of this plant in different medicines. Historically, Aegle marmelos has been used for the number of ethnobotanical purposes. At present, Aegle marmelos has become an important source of medicine for curing various human and animal diseases. Apart from exploring possibilities to prepare standardized drugs by using different plant parts of Aegle marmelos, production of jam by using its fruits should be promoted as a health tonic at commercial scale. Unfortunately, most of the compounds have not been evaluated properly for the exploration of new lead molecule. Mechanism of action of a few bioactive compounds has been identified so far. Hence, extensive research work is required to find out the mechanisms of action as well as bioactivity of the various phytochemicals and efficacy of the medicinal values of Aegle marmelos. Thus in the near future Aegle marmelos extracts could be further exploited as a source of useful phytochemical compounds and may play a very important role in modern system of medicine.
2.9 RESEARCH INTEREST IN NATURAL ANTIOXIDANTS

Antioxidant-based drugs and herbal formulations for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer’s disease and cancer have appeared during the last few decades [37]. This has now attracted a great deal of research interest in natural antioxidants from medicinal plants. A direct relationship between antioxidant activity and phenolic content of medicinal plant extracts has also been reported by scientists [47,48]. Epidemiological studies have shown that the consumption of foods and beverages rich in phenolic content can drastically reduce the incidence of cardiovascular diseases [49]. Many Indian medicinal plants are considered potential sources of phenolic compounds and also other antioxidant compounds. In some cases, their active constituents are evaluated and also well established.

There is clear and promising evidence that indigenous antioxidants may be very useful in preventing the deleterious and hazardous consequences of oxidative stress and there is also an increasing interest in the protective biochemical functions of natural antioxidants which are contained in spices, herbs, and medicinal plants [264,265]. Our attention has been focused, in particular, on the aerial parts of six Indian medicinal plants which were commonly used in the ancient days and are now forgotten.

2.10 SYNERGISTIC EFFECT OF COMPOUNDS PRESENT IN THE CRUDE EXTRACT

The medicinal activity of the formulations most probably comes from the synergistic effect of compounds present in the crude extract. According to ethno pharmacological studies the botanical remedies or therapies provide two advantages over single compound drugs. The primary active compounds in the medicinal plants are synergized by the secondary compounds and the secondary compounds ease the side effects produced by the primary active
compounds. The elaborate work of searching an ethno pharmacologically active plant extract streamlined to a single active principal ingredient may result in the failure of the potent medicinal activity of the plant. A special compound might become unstable during the extraction procedure or fractionation process or in the purified form. The fundamental basic principle for ethnopharmacology does not always exist in a single active compound but rather it is a result of the interaction of more than one active compound present in the extract. Moreover, that particular single compound might potentiate the activity and that single compound might become toxic when compared to crude plant extract. Thus, the likelihood that more than one compound present in crude plant extract could contribute to a net pharmacological response of the extract.

The complex mixtures of plant flavonoids had a synergistic effect on anti-fungal activity greater than the sum of the effects produced by their purified components.