GENERAL INTRODUCTION
MEDICINAL PLANTS:

Human beings have been utilizing plants for basic preventive and curative health care since time immemorial. Recent estimates suggest that over 9,000 plants have known medicinal applications in various cultures and countries, and this is without having conducted comprehensive research amongst several indigenous and other communities (Farnsworth and Soejarto, 1991). Medicinal plants are used at the household level by women taking care of their families, at the village level by medicine men or tribal shamans, and by the practitioners of classical traditional systems of medicine such as Ayurveda, Chinese medicine, or the Japanese Kampo system. According to the World Health Organization, over 80% of the world's population, or 4.3 billion people, rely upon such traditional plant-based systems of medicine to provide them with primary health care (Bannerman et al., 1983). Allopathic medicine too owes a tremendous debt to medicinal plants: one in four prescriptions filled in a country like the United States are either a synthesized form of or derived from plant materials (Srivastava et al., 1997). According to the World Wide Fund for Nature, the total import in 1980 of "vegetable materials used in pharmacy" by the European Economic Community was 80,738 tons (Lewington, 1993). India was the largest supplier by far, with 10,055 tons of plants and 14 tons of vegetable alkaloids and their derivatives.

It has been estimated that less than 1 – 10% of the large diversity of 2,50,000–5,00,000 plant species on the Earth have been studied chemically and pharmacologically for their medicinal properties (Farnsworth, 1991; Verpoorte, 2000). This is especially true for the tropical flora, as at date only 1% of the species in these habitats have been studied for their pharmaceutical potential (Gurib-Fakim, 2006). Tropical forests and many other tropical ecosystems are rich sources of a diversity of plant derived chemical compounds, both because of the high species diversity but also because of the "eternal summer" which forces the plant species to the constant production of chemical defense compounds against herbivores and pathogens as well as against other plant species. Plants in a tropical rainforest also have to compete for space and light and this forces species to develop more efficient means of using energy and nutrients as well as to allocating resources for secondary compound production. For these reasons a greater portion of the
tropical plant species contains secondary compounds, potentially useful as models for/as medicines (Wood-Sheldon et al., 1997). Plant derived compounds have been, and are still, important as such or as models (lead compounds) for medicines: 50% of the prescription products in various countries in Europe and the US are either natural products or natural product derivates (Cordell, 2002; Newman et al., 2003). To date about 50 drugs have come from tropical plants (Gurib-Fakim, 2006). Examples of successful medicines derived from natural product leads include most antibiotics, the acetylcholine esterase (ACE) inhibitors, many anticancer agents, the immunosuppressants, cyclosporine and rapamycin and the antiparasitic avermectins (Harvey and Waterman, 1998). With the history of utilization in both India and the rest of the world, it is not surprising that the higher plants continue to provide mankind with new remedies, and in some cases are important sources of old remedies. Van Wyk et al., (1997) reported that natural products represent more than 50% of all drugs in clinical use in the world. Well known plant derived medicines include digoxin from Digitalis spp., quinine from Cinchona spp., morphine and codeine from Papaver somniferum, atropine from Atropa belladona, and pilocarpine from Pilocarpus jaborandiz. More recently new anticancer drugs such as taxol from Taxus spp. (T. brevifolia and T. bacata) and vincristine from Catharanthus roseus have been developed. The discovery of both taxol and vincristine has demonstrated the value of plants as an important source of new molecules (Van Wyk et al., 1997).

With problems like the emergence of antibiotic resistance, plant medicines are being reexamined as a potential resource of new compounds (Cowan, 1999). Currently plants, which have been documented as traditional medicines, are being examined in the hope of finding new or improved medication. This includes research on the antimicrobial, anthelmintic, antifungal and anti-inflammatory activity of plant extracts, as well as on other aspects of systemic pharmacology (Grierson and Afolaya, 1999; Kelmanson et al., 2000; McGaw et al.; 2000; Rabe and van Staden, 1997; Shale et al., 1999). Despite certain herbal remedies being used on an extensive basis to treat various disease conditions in animals by the rural populations of India and various parts of the World, there is very little research documenting their efficacy and safety. Since their use lacks
proper scientific investigation, many of these treatments may actually not be effective or safe.

Higher plants are still poorly explored as sources of new drugs (Hostettman and Terreaux, 2000). There are several ways in selecting plant materials when searching for new medicinal plants/active compounds. Ethnopharmacological information on medicinal plants is often of substantial importance for the finding of new potential medicinal plants/new ways of using an already known plant. It has been estimated that 74% of the pharmacologically active, plant derived components were discovered after the ethnomedical uses of the plants started to be investigated (Farnsworth and Soejarto, 1991; Wood-Sheldon et al., 1997).

Traditional medicine:
Human beings must have searched from early times for substances that would relieve their suffering and cure their loved ones. Since, illness was thought to be caused by mystical agents underlying the natural world; cures for mental and physical diseases were sought among plants and animals. A perilous process of trial and error must have discovered the curative agents. Medical knowledge accumulated slowly as it was painstakingly passed on from generation to generation. Since communication between tribes was poor, remedies were probably discovered independently several times.

Plants are an integral part of many of alternative therapies such as herbalism, ayurveda, homeopathy, naturopathy, aromapathy, Unani and in other folklore systems of medicine. Undisputed documentation of the use of medicinal herbs comes only after the advent of recorded history. Sumarian drawings of opium (poppy) capsules from 2500 B.C. suggests a good knowledge of plants but substantial record of the use of herbs in medicine comes first from the code of Hammurabi, a series of tablets carved under the direction of the King of Babylon in about 1770 B.C. These tablets mention plants such as henbane, licorice and mint which are still used in medicine (Simpson and Ogorzaly, 2001). However, Dioscorides whose legacy was five-volume work entitled "De Materia Medica" made the most significant Greek contribution. This encyclopedic work
described the preparation of about 100 simple drugs. Although poorly organized and often inaccurate, it became the prototype for the present pharmacopoeias.

On the other hand, two great traditions of medicines namely the Ayurvedic Indian Medicine and the Traditional Chinese Medicine systems flourished in the East. In India, the history of medicinal use of plants has been found in Rig Veda, perhaps the oldest repository of human knowledge, which was written about 4500-1600 B.C. Further, there are comprehensive works of Charaka (100 B.C.) and Sushruta (800 B.C.), which gave a detailed description of the *Materia Medica* as it was known to ancients. The works of Atreya, Jivaka and Kahsyap (about 600 B.C.), Vyadi (about 500 B.C.), Nagarjuna (about 500 B.C.) and Patanjali (about 200 B.C.) added to the knowledge of herbal medicine.

Plants, especially medicinal plants, offer a vast resource of novel natural compounds, often with exciting activities and biological properties. Therefore, the empirical approach to discover new drugs viz., the systematic screening of plant extracts or plant derived substance still remains an interesting strategy to find new lead compounds. Structure-activity relationship studies of these leads preferentially combined with computer-graphic model building should result in molecules with optimal activity, better bio-availability, fewer side effects and an acceptable therapeutic index and consequently good candidates for the development of new drugs.

Profound changes are taking place in the medicinal thought. Today, the conventional modern allopathic medicine is not able to solve the catastrophic increase of dreadful diseases such as cancer, cardiovascular diseases, diabetes, malaria, tuberculosis, asthma, arthritis, AIDS and others yet to be understood completely. Many of these ailments are declared as incurable. But the practitioners of traditional medicine do have successful treatment for these so called incurable diseases by their age old, time tested herbal drugs.

The World Health Organization has conceptualized traditional medicine as “the basis of community’s and country’s culture, history and beliefs came into being long before the development and spread of Western medicine that originated in Europe after
the development of modern science and technology" (WHO, 2000). The knowledge of traditional medicine is often passed on verbally from generation to generation. Nevertheless, in some cases a sophisticated theory and system is involved.

The past decade has witnessed a storm of international debate and legal challenges over the patenting of traditional knowledge including its products like traditional medicines. Two confliction forces at the heart of this have been; the attempt from non-indigenous individuals and organizations to claim ownership of indigenous knowledge and commercial gain; the other has been from the indigenous groups to fend off this trend and to either take a ownership of such products themselves or to engage in partnership with fair sharing of benefits for the commercial development of their knowledge products or processes (Dutfield, 2001).

Several indigenous medicinal plants and the age-old practice of using plant since traditional systems of medicine have been indiscriminately patented to the firms of developed countries. For instance, Phyllanthus amarus, Piper nigrum and Curcuma longa used in the ayurvedic medicine since several years for treating jaundice, vitiligo (skin pigmentation disorder) and wound healing, respectively, however been patented by the firms of the US and Britain, owing to their claim of the detection of the active principle.

**Plants as antimicrobials:**
Antibiotic resistant bacteria strains with different mechanisms are found continually and thus new drugs are required (Streit *et al.*, 2004). The British National Formulary (2002) has listed 63 antibiotics that are available for the treatment of bacteria infections and many of those antibiotics are structurally related and are directed against only a few biochemical targets. For example, after the antibiotic nalidixic was discovered, it took 37 years before discovering the new antibiotic, linezolid. All other antimicrobial agents that came on market during that time period were modifications of existing molecules. Therefore, the finding of new antimicrobial agents with novel mechanisms of action is essential and extensively pursued in antibacterial drug discovery (Coates *et al.*, 2002).
Many of the drugs that are used today are related in terms of natural structure. In many cases, chemically synthesized drugs have obtained the model structure from nature. During 1981-2002, a total of 163 new chemical entities that are used as drugs were discovered. Many of those new drugs are based on natural products as a source of novel structure. Synthesized compounds have become a more interesting research area in the search for new antimicrobial agents, especially when optimizing structures to approved agents. With better techniques and knowledge, synthesized compounds will most likely also lead to better results in the future (Taylor et al., 2002; Newman et al., 2003).

Natural products, in contrast to chemically synthesized, as a source of novel antimicrobials are still common, as one-third of the best selling drugs are based on them. Natural products are still mostly an unexplored research area with a great potential for drug discovery. Plants have been the largest natural source of new drugs, though only less than 5% of known plants have been chemically characterized. Especially extracts from plants can have significant value in antimicrobial research as they may inhibit bacterial growth by different mechanisms than conventional antibiotics. For example, plant extracts that contain different phenolics have shown good antimicrobial effects and are receiving growing interest (Eloff 1998a; Cowan 1999; Rauha et al., 2000; Dorman and Deans 2000).

Conventional antibiotics and the problem of microbial resistance:
Major improvements in the early recognition and treatment of infectious diseases have been done in the last 60 years. This has resulted in a significant reduction in the morbidity and mortality associated with these diseases. Unfortunately, bacteria and fungi have developed resistance to all classes of different antibiotics discovered to date (Alanis, 2005). The use/misuse of antibiotics has led to an increasing prevalence of multiple-drug resistant (MDR) strains, and there is now an urgent need to develop new effective antibiotic agents (Cantrell et al., 2001). Serious systemic fungal and bacterial infections have become a major cause of morbidity and mortality among hospitalized patients around the world in the 21st century. The incidence of serious microbial infections is increasing due to the increasing number of immune-compromised patients due to
HIV/AIDS (Espinel-Ingroff et al., 1998). In tropical countries infectious diseases account for approximately one-half of all deaths (Iwu et al., 1999). Many bacterial diseases, which were thought to have been eradicated from Western countries, might once again become a serious health problem. There is thus an urgent need for compounds that act on novel molecular targets that circumvent the established resistance mechanisms.

Antimicrobial property of several plant products has become the part of the modern science approach to find newer drugs against the pathogenic bacteria. It has been reported that between the years 1983 and 1994, of 93 new antibacterial agents submitted to analysis by the FDA, six were natural products (teicoplanin, mupirocin, miokamycin, carumonam, isepamicin and RV-11). The systematic screening of antibacterial plant extracts represents continuous efforts to find newer compounds with the potential to act against multi-drug-resistant bacteria (Cragg et al., 1997). The continuous development of antibiotically resistant strains of microbial pathogens such as MRSA (Methicillin resistant Staphylococcus aureus), PRSP (Penicillin resistant Streptococcus pneumoniae) and VRE (Vancomycin resistant Enterococci) is a growing problem, and it is therefore, extremely important to discover and develop new antimicrobial compounds (Tally, 1999). The screening of plant extracts for antimicrobial activity such as benzoin and emetin has shown that higher plants represent a potential source of new antimicrobial compounds (Press, 1996; Cox, 1994). The antimicrobial compounds from plants may inhibit bacteria through different mechanisms than the conventional antibiotics, and could therefore be of clinical value in the treatment of microbial infections (Eloff, 1998a; Eloff, 1998b).

Staphylococcus aureus:
Staphylococcus aureus forms a fairly large yellow colony on rich medium; S. epidermidis has a relatively small white colony. S. aureus is often hemolytic on blood agar; S. epidermidis is non hemolytic. Staphylococci are facultative anaerobes that grow by aerobic respiration or by fermentation that yields principally lactic acid. The bacteria are catalase-positive and oxidase-negative. S. aureus can grow at a temperature range of 15 to 45°C and at NaCl concentrations as high as 15 %. Nearly all strains of S. aureus produce the enzyme coagulase; nearly all strains of S. epidermidis lack this enzyme.
S. aureus should always be considered a potential pathogen and most strains of S. epidermidis are nonpathogenic and may even play a protective role in their host as normal flora. *Staphylococcus epidermidis* may be a pathogen in the hospital environment.

**Methicillin-susceptible Staphylococcus aureus (MSSA):**

*Staphylococcus aureus* is the second most common microorganism isolated from infections in NICUs (Stoll *et al.*, 1996); methicillin-susceptible samples being the most common (Siegel, 1998). Such infections predominantly involve sepsis, pneumonia, cutaneous infection and conjunctivitis. MSSA infections are more prevalent in neonates, especially those with the following risk factors like premature birth, low weight, breathing syndromes, immunodeficiency, antimicrobial use, prolonged hospital stay, invasive methods and surgical interventions (Escobar *et al.*, 2000).

The epidemiology of and risk factors for healthcare associated infections caused by methicillin-resistant *S. aureus* (MRSA) have been comparatively well-studied and less is known about methicillin-susceptible *S. aureus* (MSSA). Though MSSA is generally considered an endemic pathogen, there are reports of clusters or mini-epidemics of disease among hospitalized neonates. *S. aureus* can also be carried in the nose and/or rectum of staff (Kluitmans *et al.*, 1997). Overcrowding in neonatal nurseries has been shown to be a major factor in transmission of organisms among infants. There has been little study of mixed outbreaks of MRSA and MSSA (Aires de Sousa and Conceicao, 2005) and infection control strategies to control these outbreaks. Because several infection control strategies are normally instituted simultaneously in order to halt the epidemic as soon as possible, the relative importance of specific measures is unclear. There was no evidence of any relationship between colonization and changes in either birth rate, new intakes of clinical staff or repairs and maintenance to the old building. However, infants were normally only cultured if they were thought to have a clinical infection, so colonization rates were probably underestimated (Stark and Harrisson, 1992). Although methicillin-susceptible *S. aureus* (MSSA) continues to be the most frequent pathogen in hospitalized neonates, the resistant phenotype (MRSA) is of great importance, as it is associated with many outbreaks (Davies *et al.*, 1987; Corbell *et al.*, 1997).
Mechanisms of antibiotic resistance:

*Staphylococcal* resistance to penicillin is mediated by penicillinase (a form of β-lactamase) production, an enzyme which breaks down the β-lactam ring of the penicillin molecule. Penicillinase-resistant penicillins such as methicillin, oxacillin, cloxacillin, dicloxacillin and flucloxacillin are able to resist degradation by *Staphylococcal* penicillinase.

The mechanism of resistance to methicillin is by the acquisition of the mecA gene, which codes for an altered penicillin-binding protein (PBP) that has a lower affinity for binding β-lactams (penicillins, cephalosporins and carbapenems). This confers resistance to all β-lactam antibiotics and obviates their clinical use during MRSA infections.

Glycopeptide resistance is mediated by acquisition of the vanA gene. The vanA gene originates from the Enterococci and codes for an enzyme that produces an alternative peptidoglycan to which vancomycin will not bind.

*S. aureus* has become resistant to many commonly used antibiotics. The β-lactamase-resistant penicillins (methicillin, oxacillin, cloxacillin and flucloxacillin) were developed to treat penicillin-resistant *S. aureus* and are still used as first-line treatment. Methicillin was the first antibiotic in this class to be used (it was introduced in 1959), but only two years later, the first case of methicillin-resistant *S. aureus* (MRSA) was reported. Despite this, MRSA generally remained an uncommon finding even in hospital settings until the 1990s when there was an explosion in MRSA prevalence in hospitals where it is now endemic (Johnson *et al.*, 2001).

MRSA infections in both the hospital and community setting are commonly treated with non-β-lactam antibiotics such as clindamycin (a lincosamine) and co-trimoxazole (also commonly known as trimethoprim/sulfamethoxazole). Resistance to these antibiotics has also led to the use of new, broad-spectrum anti-Gram positive antibiotics such as linezolid because of its availability as an oral drug. First-line treatment for serious invasive infections due to MRSA is currently glycopeptide antibiotics.
(vancomycin and teicoplanin). There are number of problems with these antibiotics, mainly centred around the need for intravenous administration (there is no oral preparation available), toxicity and the need to monitor drug levels regularly by means of blood tests. There are also concerns that glycopeptide antibiotics do not penetrate very well into infected tissues (this is a particular concern with infections of the brain and meninges and in endocarditis). Glycopeptides must not be used to treat methicillin-sensitive S. aureus as outcomes are inferior (Blot et al., 2002).

**Methicillin-resistant *Staphylococcus aureus* (MRSA):**

During the early part of the 20th century, fewer than 45% of people lived to the age of 65. Until the mid-20th century, infectious diseases were the leading cause of death. Despite Alexander Fleming's serendipitous discovery in 1928 of the first bactericidal antibiotic, it was not until the early 1940s that penicillin was actually produced and used to treat infectious diseases—including infections caused by *Staphylococcus aureus*.

Just a decade later, a resistant strain of *S. aureus* emerged. It was resistant not only to penicillin, but the new antibiotic arsenal as well as erythromycin, streptomycin, and tetracycline. It was 1955, and “modern medicine” was unable to effectively treat the new strain. Faced with this challenge, scientists and health care professionals continued to work collaboratively to control the transmission of the resistant *Staphylococcus* strain and find a cure. By 1960, methicillin was the newest, most effective weapon against *S. aureus* (Fig. 1). In the late 1970s, hospitals in Eastern Australia saw the first outbreaks of methicillin-resistant *Staphylococcus aureus* (MRSA). By the 1980s, MRSA had emerged in various places throughout the world (www.prhi.org).

Methicillin, cloxacillin and flucloxacillin are penicillinase-stable beta-lactam antibiotics. *Staphylococcus aureus* strains which are resistant to these drugs are referred to as methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA has been known since the 1960's. This was soon after the introduction of methicillin for clinical use. Certain strains of MRSA were found to have the propensity to spread very quickly in hospitals. These are referred to as “epidemic” strains or EMRSA. Remarkable ability to develop resistance to a variety of antibiotics including penicillins, cephalosporins, amino-
glycosides, macrolides and quinolones. This poses a major threat to public health. Concern about MRSA is related to its potential for nosocomial transmission and the limited number of antibiotics available for its treatment. According to an Indian study, the MRSA prevalence has increased from 12% in 1992 to 80.83% in 1999 (Verma et al., 2000).

Figure 1: 20,000 times magnified MRSA (Source: http://www.webmd.com)

Types of Staphylococcus aureus infections:
There is no specific 'MRSA disease' like with tuberculosis or typhoid. S. aureus infects a range of tissues and body systems giving general often ambiguous symptoms that are common to different infections caused by other bacteria.

Wound infections:
S. aureus/MRSA is the commonest cause of wound infection - either after accidental injury or surgery. This shows as a red, inflamed wound with yellow pus seeping from it. The wound may break open or fail to heal and a wound abscess could develop.
Superficial ulcers:
Pressure ulcers, varicose ulcers and diabetic ulcers (all due to poor blood supply and superficial skin damage) are often sites of MRSA infection.

Intravenous line infections:
MRSA may infect the entry site of an intravenous line causing local inflammation with pus from which the MRSA can enter the blood stream to cause a bacteraemia (blood stream infection).

Deep abscesses:
If MRSA (or any S. aureus) spreads from a local site into the blood stream it can lodge at various sites in the body (e.g. lungs, kidneys, bones, liver, spleen) and cause one or more deep abscesses distant from the original site. These can be painful with high fever, a high white cell count in the blood and signs of inflammation near the infection. The patient will be very unwell and may have rigors (shivers) and low blood pressure (shock). Over a period, the body enters a catabolic state with breakdown of tissue, loss of weight and failure of essential organs. This is usually linked with an associated septicemia.

Lung infections:
S. aureus/MRSA is a rare cause of lung infection except in Intensive Care Units. There, the patient is on a ventilator with a tube in the trachea, bypassing the defenses of the nose and throat. MRSA can gain entry to the lungs via the tube and cause pneumonia which may be fatal.

Bacteraemia / septicaemia:
S. aureus/MRSA can enter the normally sterile blood stream either from a local site of infection (wound, ulcer, abscess) or via an intravenous catheter (placed there for their medical care). Bacteraemia describes the presence of S. aureus/MRSA in the blood. Septicaemia can follow and is the clinical term for a severe illness caused by the bacteria in the blood stream. The symptoms are not specific to MRSA and can be the same for other bacteria that cause septicaemia. Typically symptoms can include high fever, raised white cell count; rigors (shaking), disturbance of blood clotting with a tendency to bleed
and failure of vital organs. This is the kind of MRSA infection that has the highest death rate.

**Endocarditis:**
The incidence of *S. aureus* endocarditis has increased and now accounts for 25 to 35% of cases (Sanabria et al., 1980; Sandre and Shafran, 1996). It occurs in intravenous drug users, elderly patients, patients with prosthetic valves, and hospitalized patients. In all four groups, the initial presentation may be limited to fever and malaise, making diagnosis difficult. Unlike endocarditis caused by less virulent pathogens, *S. aureus* endocarditis is characterized by a rapid onset, high fever, frequent involvement of normal cardiac valves, and the absence of physical stigmata of the disease on initial presentation (Chambers et al., 1983).

**Toxic Shock Syndrome:**
*Staphylococcal* toxic shock syndrome came to prominence in 1980-1981, when numerous cases were associated with the introduction of superabsorbent tampons for use during menstruation. The disease is characterized by a fulminant onset, often in previously healthy persons. The diagnosis is based on clinical findings that include high fever, erythematous rash with subsequent desquamation, hypotension, and multiorgan damage. Alternative diagnoses, including Rocky Mountain spotted fever, Streptococcal scarlet fever, and leptospirosis, must be ruled out. The toxic shock syndrome often develops from a site of colonization rather than infection (Chesney et al., 1981).

**MRSA significance in HIV infections:**
HIV-infected subjects have high incidence rates of *S. aureus* infections (Witt et al., 1987; Jacobson et al., 1998) probably due to the high burden of colonization (McDonald et al., 2003) to behavioral risk factors (Mathews et al., 2005) and to frequent health care facility exposures (Onorato et al., 1999). In the clinical management of HIV-infected patients, especially in those admitted to hospital for clinical sepsis, and/or skin and soft tissue infections, clinicians should be aware of the risk factors associated with a MRSA infection, particularly low CD4 cell count, longer previous hospital stay and previous invasive procedures.
Metastatic Infections

*S. aureus* has a tendency to spread to particular sites, including the bones, joints, kidneys, and lungs (Musher et al., 1994; Libman and Arbein, 1984). Supportive collections at these sites serve as potential foci for recurrent infections. Patients with persistent fever despite appropriate therapy should be evaluated for the presence of supportive collections.

Treatment for MRSA:
Currently, microbiology laboratories should be routinely testing for susceptibility to macrolides, clindamycin, and trimethoprim-sulfamethoxazole (TMP-SMX) in addition to beta-lactam antibiotics. Most Community Associated Methicillin resistant *S. aureus* (CA-MRSA) isolates are resistant to macrolides, but are sensitive to clindamycin and trimethoprim-sulfamethoxazole. These two antibiotics have become increasingly important in the management of *Staphylococcal* infections.

A beta-lactam antibiotic such as dicloxacillin or cephalaxin had previously been considered the initial antibiotic of choice for empiric treatment of outpatient skin and soft-tissue infections. Similarly, nafcillin, oxacillin, or cefazolin had been the agents of choice for initial treatment of moderate to severe infections such as osteomyelitis, septic arthritis, or pneumonia with empyema. However, these agents are no longer appropriate when CA-MRSA is a consideration.

Vancomycin should be included in the initial antibiotic regimen for life-threatening infection when a likely pathogen is *S. aureus*. Published experience with use of clindamycin for treatment of MRSA infections is limited. Many healthcare-associated *S. aureus* infections are resistant to clindamycin. Clindamycin treatment failures are well reported, especially for infective endocarditis. Also, it should be noted that the oral suspension of clindamycin has a particularly poor taste. This results in a high rate of medication refusal by infants and young children.

Many experts currently recommend TMP-SMX for treatment of skin and soft-tissue infections in regions where MRSA isolates account for at least 15% of infections (Kaplan, 2005). This approach would minimize the likelihood of promoting resistance to
clindamycin, which is an important antibiotic for treating osteomyelitis, septic arthritis, and pleural empyema caused by MRSA (Frank et al., 2002; Martinez-Aquilar et al., 2003). A number of studies have shown the effectiveness of TMP-SMX in treating Staphylococcal infections, although most of these pathogens were methicillin sensitive (Ardati et al., 1979; Adra and Lawrence, 2004).

Plants with anti-Staphylococcus aureus activity:
To minimize the increasing rate of resistance throughout the time, it is a necessity to have continuous research for new, safe and effective anti-Staphylococcus aureus as alternative agents to rotate or replace with no effective ones. Plants and microorganisms are potent candidates for this purpose. In this regard many workers have reported anti-Staphylococcus aureus activity from plant origins. Mc Cuitecheon et al. (1992) tested 100 methanolic extracts of plants by British Colombian Native people, against 11 bacterial isolates. They found 85% of the plants were active at least against one of the bacteria. In order to find new antibacterial agents Mansouri (1999) tested ethanol extracts of 10 plants against 489 samples of S. aureus. From the plant extracts screened for antibacterial activity, Myrtus communis L. (leaves) had the greatest activity, inhibiting the growth of 99% of the isolates. Glycyrrhiza glabra L., Eucalyptus globolus Labill and Menta viridis L., were also active against the isolates inhibiting the growth of 90, 59.5 and 48.7% of the isolates, respectively. All of these extracts were active against the reference strains of S. aureus tested. Saturia hortensis L., Teucrium polium L., and Achillea santolina L., had very little antibacterial activity, while Trigonella foenum graecum L., Echium amoenum Fisch and Mey (flowers) and Juglans regia L. (leaves), had no antibacterial activity against the bacterial isolates (Mansouri, 1999). Terminalia avicennioides, Phyllanthus discoideus, Bridella ferruginea, Ageratum conyzoides, Ocimum gratissimum and Acalypha wilkesiana plants were reported to possess anti-methicillin Resistant Staphylococcus aureus (MRSA) activity (Akinyemi et al., 2005). Excoecaria cochinchinensis Lour, Salvia officinalis Lour and Argyreia nervosa (Burm.f) Bojer, leaves were reported as anti-Staphylococcus aureus activity. Antibiotic activity via multidrug efflux mechanisms of Southern prickly ash bark, Zanthoxylum clava-herculis and its compound was demonstrated by Gibbons et al. (2003). The plant species belong
to Rhus genus is also reported for their potent anti-Staphylococcus aureus activity (Rayne and Mazza, 2007).

Alternative therapies for staph infection are meant to strengthen the immune system and prevent recurrences. Among the therapies believed to be helpful for the person with a staph infection are yoga (to stimulate the immune system and promote relaxation), acupuncture (to draw heat away from the infection), and herbal remedies. Herbs that may help the body overcome, or withstand, staph infection include:

- Garlic (*Allium sativum*): This herb is believed to have antibacterial properties. Herbalists recommend consuming three garlic cloves or three garlic oil capsules a day, starting when symptoms of infection first appear.
- Cleavers (*Galium aparine*): This anti-inflammatory herb is believed to support the lymphatic system. It may be taken internally to help heal staph abscesses and reduce swelling of the lymph nodes. A cleavers compress can also be applied directly to a skin infection.
- Goldenseal (*Hydrastis canadensis*): Another herb believed to fight infection and reduce inflammation, goldenseal may be taken internally when symptoms of infection first appear. Skin infections can be treated by making a paste of water and powdered goldenseal root and applying it directly to the affected area. The preparation should be covered with a clean bandage and left in place overnight.
- Echinacea (*Echinacea* spp.): Taken internally, this herb is believed to have antibiotic properties and is also thought to strengthen the immune system.
- Thyme (*Thymus vulgaris*), lavender (*Lavandula officinalis*), or bergamot (*Citrus bergamot*) oils: These oils are believed to have antibacterial properties and may help to prevent the scarring that may result from skin infections. A few drops of these oils are added to water and then a compress soaked in the water is applied to the affected area.

Tea tree oil (*Melaleuca* spp.) another infection-fighting herb and this oil can be applied directly to a boil or other skin infection. Shahidi Bonjar (2004) investigated 180 methanolic plant extracts of 72 families against three isolates of *S. aureus* in an in vitro
bioassay and reported that 79 plant samples in 41 families exhibited anti-

Plants as antioxidants:
Free-radical reactions have been implicated in the pathology of many human diseases/disease (Table.2) conditions like atherosclerosis, ischemic heart disease, aging process, inflammation, diabetes, immune-suppression, neurodegenerative diseases etc. (Maxwell, 1995; Droge, 2002; Beckman and Ames, 1998). Radicals and other reactive oxygen species are formed constantly in the human body and are removed by the enzymic and non-enzymic antioxidant defense systems (Halliwell and Gutteridge, 1989). The disturbance in 'redox homeostasis' occurring when antioxidant defenses are inadequate can damage lipids, proteins, carbohydrates, and DNA. Drugs with multiple protective mechanisms, including antioxidant activity, may be one way of minimizing tissue injury (Halliwell, 1991).
Oxidation process is one of the most important routes for producing free radicals in food, drugs and even living systems. Catalase and hydroperoxidase enzymes convert hydrogen peroxide and hydroperoxides to nonradical forms and function as natural antioxidants in human body. Due to depletion of immune system natural antioxidants in different maladies, consuming antioxidants as free radical scavengers may be necessary (Halliwell, 1994; Kuhnan, 1976; Kumpulainen and Salonen, 1999; Younes, 1981). Currently available synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinone 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. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity (Barlow, 1990; Branen, 1975). Knowledge on the protective mechanisms against toxin and drug induced organ-toxicities leads scientists to look for biologically active relevant drugs from herbal plants, which can possess intrinsic antioxidant activity and protect those organs from unwanted oxidative stress. In the modern medicine, plants occupy a significant birth as raw materials for some important drug preparations (de Mejia and Ramirez-Mares, 2002; Iwu et al., 1994; Chopra et al., 1986). India is well known for a plethora of medicinal plants. The traditional Indian medicinal plants act as antiradicals and DNA cleavage protectors (Russo et al., 2001). These plants have also been considered to protect health, longevity, intelligence, immunosurveillance and body resistance against different infections and diseases.

In recent years, the use of natural antioxidants present in food and other biological materials has attracted considerable interest due to their presumed safety, nutritional and therapeutic value (Ajila et al., 2007). Nutraceuticals are supposed to hold the key to a healthy society in the coming future. Antioxidants derived from fruits, vegetables, herbs, spices and cereals are very effective and have reduced interference with the body’s ability to use free radicals constructively (Kahkonen et al., 1999; Wolfe et al., 2003). Natural antioxidants mainly come from plants in the form of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols) ascorbic acid and carotenoids. The quest for natural antioxidants for dietary, cosmetic and pharmaceutical
uses has become a major industrial and scientific research challenge over the last two decades. Efforts to gain extensive knowledge regarding the power of antioxidants from plants and to tap their potential are therefore on the increase.

In developing countries like India where poverty and malnutrition is rampant, knowledge of plant derived antioxidants could reduce the cost of health care. India has a rich history of using various herbs and herbal components for treating various diseases. Many Indian plants have been investigated for their beneficial use as antioxidants or source of antioxidants using presently available experimental techniques. Scartezzini and Speroni (2000) have reviewed extensively about Curcuma longa, Magnifera indica, Momordica charantia, Phyllanthus emblica, Santalum album, Swertia chirata, Withania somnifera that have antioxidant activity and used in Indian traditional medicine. Recently Govindarajan et al., (2003) has reviewed Acorus calamus, Aloe vera, Andrographis paniculata, Asparagus racemosus, Azadirachta indica, Bacopa monnieri, Desmodium gangeticum, Glycyrrhiza glabra, Picrorhiza kuruoa, Psoralea corylifolia, Semecarpus anacardium, Terminalia chebula, Tinospora cordifolia. Jain et al., (2008) reported the antioxidant activity of Momordica dioica against CCl₄ toxicity in albino rats. Apart from these plants, numerous other plants used in Indian traditional medicine are reported to show antioxidant activity.

Oxidative stress:
Oxidative stress has been defined as “a disturbance in the pro-oxidant/antioxidant balance in favour of the former, leading to potential damage” (Sies, 1991). Mammalian cells generate reactive oxygen species (ROS) during normal metabolic processes. The cell has several ways to respond to ROS. It can either repair and remove the damaged nucleotides and lipid peroxidation by-products or directly reduce the ROS via enzymatic and non-enzymatic antioxidants.

 Reactive oxygen species:
The incomplete reduction of molecular oxygen during cellular metabolism or spontaneously by auto-oxidation reactions in the environment can result in formation of reactive oxygen intermediates (ROIs) such as (Table.1) superoxide radicals (O₂⁻),
hydrogen peroxide (H₂O₂), hydroxyl radicals (OH⁻) and singlet oxygen (¹⁰₂) (Fridovich, 1978). These ROI’s can damage cell components such as DNA, RNA, protein and lipids.

**Molecular oxygen**

Theoretically, O₂ should be an excellent terminal electron acceptor because the E’o of the O₂/H₂O half-cell system is very high (+0.8 V at pH 7.0). Oxygen in its ground state is a non-toxic triplet inorganic molecule which has one unpaired electron in each of its two π* outer antibonding orbitals. However, due to the parallel directions of spin of these electrons, molecular oxygen cannot always accept two electrons readily from a reduced molecule. O₂ must accept a pair of electrons having a spin direction opposite to that of the two unpaired electrons of the O₂ molecule, thus obeying the Pauli exclusion principle (Martinez-Cayuela, 1995). This requirement restricts the range of compounds oxidized by oxygen (Farr and Kogoma, 1991). The alternative to spontaneous two-electron reduction is a one-electron reduction that leads to formation of O₂⁻. The reduction of O₂ to H₂O as the terminal reaction of an electron transport system requires four electrons (O₂ + 4e⁻ + 4H⁺ → 2 H₂O) and does not generate O₂⁻. However, partial reduction of O₂ can generate ROIs, as indicated below (Salin and Brown-Peterson, 1993):

\[
\begin{align*}
O₂ + 1e^- & \rightarrow O₂^- . \\
O₂ + 2e^- + 2H^+ & \rightarrow H₂O₂ \\
O₂ + 3e^- + 3H^+ & \rightarrow OH^- + H₂O
\end{align*}
\]

**Singlet oxygen (¹⁰₂)**

Singlet oxygen is an energized form of O₂ in which the direction of spin of one unpaired electron of ground-state dioxygen is reversed by an input of energy. This can give rise to either of two forms of singlet oxygen: O₂ (¹Σg), in which the two electrons continue to occupy separate orbitals and O₂ (¹Δg), in which the two electrons occupy one orbital and neither occupies the other orbital. Singlet oxygen is not a radical therefore does not possess unpaired electrons. Singlet oxygen is highly reactive because the spin restriction associated with ground state O₂ has been removed. It can subsequently oxidize a large variety of biological molecules such as lipids, proteins, and DNA and is responsible for
cell destruction caused by light and some photosensitizers (Weters, 1987; Sies and Menck, 1992). \( ^{1}O_{2} \) can be formed in a number of chemical, photochemical, and biochemical systems involving photo oxidations, free radicals and lipid peroxides (Murray, 1979).

The superoxide radical (\( O_{2}^{-} \))

The univalent reduction of molecular oxygen produces the superoxide radical, which has one unpaired electron. Superoxide radicals exhibit moderate reactivity towards biomolecules in an aqueous environment compared to other ROIs and are capable of acting as either a reductant or oxidant. This moderate activity allows \( O_{2}^{-} \) to diffuse for relatively long distances in biologic systems and thus can be generated at sites distant to the site at which it eventually causes toxicity (Miller and Britigan, 1995). Superoxide can be generated enzymatically by certain flavoprotein dehydrogenases or non-enzymatically through the autooxidation of molecules such as ferredoxins, hydroquinones, and thiols (Fridovich, 1978; Salin and Brown-Peterson, 1993). The superoxide radical has been reported to exert a direct effect on certain enzymes such as catalase (Kono and Fridovich, 1982), aconitase (Gardner and Fridovich, 1992) and glutathione peroxidase (Blum and Fridovich, 1985). However its main role in oxygen toxicity is probably due to its dismutation to form \( H_{2}O_{2} \) or its interaction with \( H_{2}O_{2} \) in an iron catalyzed Haber-Weiss reaction which can produce reactive hydroxyl radicals (Salin and Brown-Peterson, 1993). The dismutation to \( H_{2}O_{2} \) occurs when one \( O_{2}^{-} \) gives up its electron to another \( O_{2}^{-} \) as follows:

\[
O_{2}^{-} + O_{2}^{-} + 2H^{+} \rightarrow H_{2}O_{2} + O_{2}
\]

Superoxide radicals will dismutate spontaneously but the reaction is limited by the electrostatic repulsion of the two anions (Fridovich, 1978). At pH 13.0 superoxide radicals have a half-life of about 160 min whereas at pH 7.0 it is approximately a millisecond. The half-life is about 100 times less at pH 4.8, which is the pK value, where equal concentrations of the ionized and nonionized forms are present (\( O_{2}^{-} \) and \( HO_{2} \)):

\[
HO_{2} + O_{2}^{-} \rightarrow HO_{2}^{-} + O_{2}
\]
At pH 4.8 there is no charge repulsion, and dismutation takes place faster. The rate actually decreases from pH 4.8 to pH 2.0 and then remains constant below pH 2.0. The decrease occurs because the reaction

\[ \text{H}_2\text{O}_2 + \text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]

is slower than

\[ \text{H}_2\text{O}_2 + \text{O}_2^- \rightarrow \text{H}_2\text{O}_2^- + \text{O}_2 \]

The iron catalyzed Haber-Weiss reaction occurs in two steps as follows:

\[ \text{O}_2^- + \text{Fe}^{3+} \text{chelate} \rightarrow \text{O}_2 + \text{Fe}^{2+} \]

\[ \text{Fe}^{2+} \text{chelate} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} \text{chelate} + \text{OH}^- + \text{OH}^- \]

\[ \text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \text{OH}^- + \text{OH}^- + \text{O}_2 \]

In this series of reactions, \( \text{O}_2^- \) acts as a reducing agent for the iron in \( \text{Fe}^{3+} \) chelate. Other reducing agents can accomplish the same reduction and thus superoxide radicals are not absolutely necessary for the generation of hydroxyl radicals. The second step, in which ferrous ions (produced from ferric ions by whatever mechanism) reduce \( \text{H}_2\text{O}_2 \) is called the Fenton reaction.

**Hydrogen peroxide (H\(_2\)O\(_2\))**

The most stable of the oxygen intermediates is hydrogen peroxide, which is not a free radical. It results from the addition of two electrons to \( \text{O}_2 \) or as a product of dismutation of superoxide radicals. \( \text{H}_2\text{O}_2 \) is a more reactive oxidant than \( \text{O}_2^- \) and, being uncharged and soluble in organic solvents, it readily crosses biological membranes. The reactions of \( \text{H}_2\text{O}_2 \) with organic molecules remain unclear, partly because it reacts quickly in the presence of contaminating metals to form other ROIs which obscure its own role in oxidation reactions (Farr and Kogoma, 1991). It can act as a weak oxidizing agent and can damage DNA (Steiner et al., 1984), lipids (Kellogg and Fridovich, 1977) and can attack thiol groups of proteins or reduced glutathione. It can also react directly with some keto acids (Halliwell and Gutteridge, 1990; Wefers and Sies, 1983). Most importantly, \( \text{H}_2\text{O}_2 \) will react with reduced iron or copper ions to generate hydroxyl radicals (OH') in the Fenton reaction (Cadenas, 1989).
Certain reactions catalyzed by flavoproteins such as xanthine oxidase or NADPH oxidase generates \( \text{H}_2\text{O}_2 \) by forming \( \text{O}_2^- \) as an intermediate, which can then dismutate. The \( \text{O}_2^- \) is generated when the reduced prosthetic group, \( \text{FADH}_2 \) reacts spontaneously with two molecules of \( \text{O}_2 \).

\[
\text{FADH}_2 + 2 \text{O}_2 \rightarrow 2 \text{O}_2^- + \text{FAD}
\]

The \( 2 \text{O}_2^- \) then undergo dismutation to yield \( \text{O}_2 \) and \( \text{H}_2\text{O}_2 \). In other oxidase reactions, however, \( \text{H}_2\text{O}_2 \) can be generated directly by a two-electron reduction of \( \text{O}_2 \) without formation of \( \text{O}_2^- \) as an intermediate (Salin and Brown-Peterson, 1993).

\[
\text{FADH}_2 + \text{O}_2 \rightarrow \text{H}_2\text{O}_2 + \text{FAD}
\]

Both \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) can also be generated nonenzymatically during the autooxidation of various reduced flavins, quinones, thiols, and iron/sulfur proteins (Fridovich, 1978; Misra and Fridovich, 1971).

**The hydroxyl radical (OH')**

Hydroxyl radicals result from the univalent reduction of \( \text{H}_2\text{O}_2 \). Hydroxyl radicals are extremely powerful oxidants (the \( E'(pH \ 7.0) \) of the reaction \( \text{OH}^- + e^- \rightarrow \text{OH}^- \) is +2.33 V) and have the potential to cause oxidative damage to almost any cell component. Hydroxyl radicals have a short half-life in solution since they react with other molecules at nearly diffusion controlled rates. The main source of hydroxyl radicals is the metal-catalyzed Haber-Weiss reaction as described above (Martinez-Cayuela 1995).

**Sources of reactive oxygen species:**

Free radicals and various reactive oxygen species are continuously produced in the body (Halliwell and Chirico, 1993; Rice-Evans and Burdon, 1993; Halliwell, 1994; Halliwell et al., 1995). They can be formed as a by-product in the mitochondrial respiratory chain due to leakage of electrons from the electron transport chain or by reactions catalysed by transition metal ions such as iron and copper ions. They may also be derived from external sources such as cigarette smoke, radiation, UV light, pollution and from the metabolism of certain drugs. The free radicals formed can react with DNA, proteins and
lipids in the body and cause extensive oxidative damage (Halliwell and Chirico, 1993; Rice-Evans and Burdon, 1993; Halliwell, 1994; Halliwell et al., 1995).

Free radicals are not only produced as an unwanted product; they are also formed deliberately in the body for useful purposes and have important physiological functions (Halliwell and Chirico, 1993; Rice-Evans and Burdon, 1993; Halliwell, 1994; Halliwell et al., 1995). A well-defined role for free radicals is when activated phagocytic cells (neutrophils, monocytes, macrophages and eosinophils) produce superoxide anion radicals and hydrogen peroxide as one mechanism to kill bacteria and fungi and to inactivate viruses (Curnutte and Babior, 1987). In addition, free radicals are also produced by an array of enzymes e.g. pyruvate metabolising enzymes, oxidases, carboxylases, hydroxylases, peroxidases, fruit ripening enzymes and radical enzymes (Halliwell and Gutteridge, 1999).

Table 1: Types of reactive oxygen species.

<table>
<thead>
<tr>
<th>ROS</th>
<th>Types</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radicals</td>
<td>Superoxide</td>
<td>O₂⁻</td>
</tr>
<tr>
<td></td>
<td>Hydroxyl</td>
<td>'OH</td>
</tr>
<tr>
<td></td>
<td>Alkoxyl</td>
<td>LO/RO⁻</td>
</tr>
<tr>
<td></td>
<td>Peroxyl</td>
<td>LOO⁻ /ROO⁻</td>
</tr>
<tr>
<td></td>
<td>Nitric oxide</td>
<td>NO⁻</td>
</tr>
<tr>
<td></td>
<td>Thyl radical</td>
<td>R-S</td>
</tr>
<tr>
<td>Non-radicals</td>
<td>Hydrogen peroxide</td>
<td>H₂O₂</td>
</tr>
<tr>
<td></td>
<td>Hypochlorous acid</td>
<td>HOCl</td>
</tr>
<tr>
<td></td>
<td>Ozone</td>
<td>O₃</td>
</tr>
<tr>
<td></td>
<td>Singlet oxygen</td>
<td>'O₂</td>
</tr>
<tr>
<td></td>
<td>Peroxynitrite</td>
<td>ONOO⁻</td>
</tr>
<tr>
<td></td>
<td>Lipid peroxide</td>
<td>LOOH</td>
</tr>
</tbody>
</table>
Exogenous sources
- γ irradiation
- UV irradiation
- Ultrasound
- Food
- Drugs
- Pollutants
- Xenobiotics
- Toxins

Endogenous sources
- Cells (e.g., neutrophils)
- Direct-producing ROS Enzymes (e.g., NO synthase)
- Indirect-producing ROS enzymes (e.g., xanthin oxidase)
- Metabolism (e.g., mitochondria)
- Diseases (e.g., metal disorders, ischemic processes)

Figure 2: Exogenous and endogenous sources of reactive oxygen species (ROS).
(Adapted from: Kohen and Nyska, 2002)

Oxidative-damage targets and types:
The continuous efflux of ROS from endogenous and exogenous sources (Fig. 2) results in continuous and accumulative oxidative damage to cellular components (Comporti, 1989) and alters many cellular functions (Gracy et al., 1999). Among the biological targets most vulnerable to oxidative damage are proteinaceous enzymes (Halliwell and Gutteridge, 1999, Levine and Stadtman, 2001), lipidic membranes (Davis, 1987; Halliwell and Gutteridge, 1999), and DNA (Beckman and Ames, 1997; Halliwell and Gutteridge, 1999).

Lipid peroxidation:
The process of lipid peroxidation involves a set of chain reactions (Fig.3) that are initiated by the abstraction of a hydrogen atom from the carbon in an unsaturated acyl
chain by ·OH. Then the carbon-centered lipid radical (L·) is oxidized by oxygen and forms lipid peroxyl radical (LOO·). Lipid peroxyl radical can propagate the peroxidation chain reaction and cause another abstraction of hydrogen from other vicinal unsaturated fatty acids. The series of chain reactions can spread into remote sites (Southorn and
Powis, 1988), resulting in the production of several alkanes, hydroxyl, epoxy derivatives, alcohols, ketones and aldehydes (e.g. malondialdehyde).

Lipid peroxidation can alter the fluidity, selective permeability and under extreme circumstances, the integrity of cell membranes, thus affecting the viability of cells. The end products of lipid peroxidation, such as water-soluble aldehydes can act as cross linking agents causing protein to aggregate; one example is the formation of age pigment, lipofuscin (Davis, 1987). Lipid peroxidation products also cause the inhibition of protein synthesis (Fraga et al., 1989), alterations in enzyme functions and they can react with nitrogenated bases of DNA (Park and Floyd, 1992), giving rise to mutations and altered patterns of gene expression (Demple et al., 1986).

**Protein modifications:**

When exposed to free radicals, proteins undergo oxidative modification especially on the Tyr, Phe, Trp, His, Met and Cys residues (Davies et al., 1987). These alterations include the scission of the peptide backbone through the peroxyl radical mediated α-carbon-nitrogen bond cleavage (Schuessler and Schilling, 1984), free radical mediated cross-linking and oxidative glycosylation etc. (Hunt et al., 1988).

Structural changes of the proteins, particularly the increase in hydrophobicity are sufficient to activate proteinases which are otherwise latent (Davis and Goldberg, 1987) and the cells undergo proteolytic burst. The protein degradation process involves a large enzyme complex of 650 KDa known as macro oxyproteina$e (MOP) (Pacifici et al., 1989). The proteolytic degradation process of modified proteins can prevent not only their accumulation and aggregation but also recycle aminoacids for de novo synthesis of new proteins.

**DNA damage:**

Nucleic acids are also targets of ROS. It is shown that mitochondrial DNA (mt DNA) is more susceptible to ROS attack than nucleic DNA (Agarwal and Sohal, 1994, Yakes and van Houten, 1997) because ROS ar mainly generated in mitochondria and mt DNA is not shielded by histone proteins as is the case for nuclear DNA. The nucleotide bases in DNA
molecules, especially the pyrimidines, are vulnerable to free radical attack. Alterations in the pyrimidine ring results in local distortions of the double helix structure (Tice et al., 1985) and consequently causes DNA breaks, sister chromatid exchange, DNA-DNA and DNA-protein cross-linking and base modifications. The replication process can be affected by DNA damages. This occurs when the DNA polymerase encounters strand lesions and the enzyme misreads the modified genetic sequences, as a result a faulty DNA daughter strand will be generated.

8-Hydroxydeoxyguanosine (8-OHdG), one product of oxidized nucleotide is used as an indicator of DNA damages. For e.g. it has been demonstrated that both 8-OHdG level and protein carbonyl content increase with age (Sohal et al., 1995, Sohal, 1997), showing that DNA damage and protein oxidation are biochemical processes associated with aging.

Figure 4: Reactive oxygen species (ROS)-induced oxidative damage
(Adapted from: Kohen and Nyska, 2002)
Oxidative stress mediated diseases:
Recent studies have indicated that ROS play a key role in the pathophysiological pathway (Fig.4) of wide variety of clinical and experimental diseases (Baud and Ardaillon, 1986; Nath and Salahudeen, 1990). About 100 disorders like rheumatoid arthritis, hemorrhagic shock, cardiovascular diseases, cystic fibrosis, metabolic disorders, neurodegenerative disease, gastro intestinal ulcerogenesis, and AIDS have been reported as the ROS mediated disorders (Das et al., 1997; Halliwell, 1991 and 1997). Some of the examples are described below.

Aging:
Aging is a universal biological process (Lee and Wei, 2001). It is hypothesized that aging is the consequence of accumulation of ROS-induced damages during oxidative stress (Lee and Wei, 2001) and mitochondrial decay plays an important role in aging (Sohal, 1997).

ROS cause damages and hence dysfunctions to many organelles, especially the mitochondrion. Results from various studies indicated that Complexes I and IV of the mitochondrial electron transport chain showed a significant functional decline in aged mitochondria (Sugiyama et al., 1993; Takasawa et al., 1993). In addition the impairment in mitochondrial antioxidant enzymes activities may be due to the accumulation of DNA damage (Lee et al., 1999). In this regard, mitochondrial DNA mutation was found to be increased in aged tissues (Lee et al., 1994 and 1999; Wei, 1998).

Atherosclerosis:
Atherosclerosis is the narrowing the lumen of an artery caused by the formation of plaque that leads to the decrease in blood supply. It is suggested that ROS oxidize cholesterol-rich low density lipoprotein (LDL) in the artery wall and the oxidized LDL cannot re-enter the lumen since the LDL receptor cannot recognize the oxidized form of LDL. Oxidized LDL can stimulate the infiltration of monocytes into the intimal space (Palinski et al., 1989) and these cells sequester oxidized LDL to form foam cells. The accumulation of foam cells will contribute to the formation of the plaque. Atherosclerosis causes many cerebrovascular disorders such as ischemic heart disease and stroke.
Alzheimer’s disease:
Alzheimer’s disease (AD) involves the degeneration of neurons in the brain, resulting in the loss of cognitive function in the elderly. The exact mechanism causing Alzheimer’s disease is still unknown, but it is suggested that the increasing level of β-amyloid peptides which may be caused by gene mutations can lead to the formation of tau aggregate within neurons. Tau aggregates then form the plaque and tangle that cause cell death (Davies, 2000). Recently, many studies have suggested the involvement of ROS mediated processed in the pathogenesis of AD, such as the increases in mitochondrial 8-OHdG, MDA and protein carbonyl levels in postmortem AD brain specimens (Mecocci et al., 1994; Lyras et al., 1994; Beal, 1995). In addition, increased intracellular β-amyloid level was found to produce oxidative damage in Alzheimer’s disease (Ohyagi and Younkin, 1996) and antioxidant treatment could slow down the progression of Alzheimer’s disease (Sano et al., 1997).

Cancer:
The development of cancer can be viewed as a micro evolutionary process that requires the cumulative action of multiple events. In recent years, accumulated evidence has shown that free radicals are involved in carcinogenesis (Guyton and Kensler, 1993; Feig et al., 1994). Free radicals can stimulate cancer development at all stages like the induction of a DNA mutation in a somatic cell (initiation stage), the stimulation of tumorigenic expansion of the cell clone (promotion stage) and the malignant conversion of the tumor into cancer (progression stage).

Oxygen free radicals also play a role in tumor promotion. This is mediated by increasing cytosolic calcium ions through the mobilization of intracellular Ca^{2+} stores and the influx of extracellular Ca^{2+} (Larsson and Cerutti, 1990). It has been proposed that oxidative stress in tumor cell decreased genomic stability and increases the sensitivity of the tumor cell to free radicals through lowering the activity of antioxidant enzymes (Punnonen et al., 1994).
Ischemia-reperfusion injury:
Xanthine oxidase plays a critical role in generation free radicals during the ischemia-reperfusion process (Coudray et al., 1992; Sanhueza et al., 1992; Hotter et al., 1995). There are other possible mechanisms involved in the production of free radicals during the ischemia-reperfusion process. For example, at the beginning of reoxygenation, the mitochondrial respiratory chain is overloaded with electrons, leading to the generation of superoxide anion radical form oxygen (Piantadosi and Zhang, 1996; Perez-Pinzon et al., 1997).

Inflammatory diseases:
It is becoming increasingly apparent that certain types of inflammatory tissue injury are mediated by reactive oxygen metabolites. The most likely sources of these oxidizing agents are the phagocytic leukocytes (e.g., neutrophils, monocytes, macrophages, and eosinophils) that invade the tissue. These radical species can clearly degrade hyaluronic acid, modify collagen and perhaps proteoglycan structure and/or synthesis, alter and interact with immunoglobulins, activate enzymes and inactivate their inhibitors, and possibly participate in chemotaxis (Greenwald, 1991). It is becoming increasingly apparent that in addition to promoting cytotoxicity, reactive oxygen metabolites may also initiate and/or amplify inflammation via the upregulation of several different genes involved in the inflammatory response, such as those that code for proinflammatory cytokines and adhesion molecules (Conner and Grisham, 1996). Oxidative stress at sites of chronic inflammation can cause permanent genetic changes. The development of mutations in the p53 tumor suppressor gene and other key regulatory genes could help convert inflammation into chronic disease in rheumatoid arthritis and other inflammatory disorders (Paul et al., 2000).

Diabetes:
Diabetes is a major worldwide health problem predisposing to markedly increased cardiovascular mortality and serious morbidity and mortality related to development of nephropathy, neuropathy and retinopathy (Zimmet et al., 1997). Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in
the pathogenesis of both types of diabetes mellitus. Mechanisms involved in the increased oxidative stress in diabetes include not only oxygen free radical generation due to nonenzymatic glycosylation (glycation), autooxidation of glycation products, but also changes in the tissue content and activity of antioxidant defense systems. Increased levels of the products of oxidative damage to lipids have been detected in serum of diabetic patients, and their presence correlates with the development of complications (Maritim et al., 2003; Zalba et al., 2006; Brownlee, 2001; Heistad Donald, 2005; Liu et al., 2006).

Oxidative stress in bacterial infection:
Bacterial infection has been found to increase the levels of inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6 in the peripheral system and in the brain of rodents (Koedel et al., 2004; Stoycheva and Murdjeva, 2005; Turrin et al., 2001). In the meantime, bacterial infection has also been found to increase the formation of reactive oxygen species (ROS), such as oxygen ions, free radicals, and peroxides, in the liver, heart, lung, blood and brain in experimental animal models leading to oxidative stress (Sakaguchi and Furusawa, 2006; Victor et al., 2005). The inflammatory cytokines and ROS act as critical mediators of the host's immune response to fight infecting bacteria (Knight, 2000; Sikora, 2000; Slifka and Whitton, 2000). Heping Zhou (2008) reported the relationship between maternal bacterial infection and the development of cerebral palsy in the offspring, and the role of inflammatory cytokines and oxidative stress in the induction of white matter damage in the offspring following maternal bacterial infection.

Antioxidant defense systems:
All tissues and cells contain defense systems for detoxification of biological reactive molecules and to prevent or minimize cellular damage through the action of antioxidants (Fig.5). Antioxidants are substances that delay or prevent the oxidation of cellular substrates and can be divided into two categories.

1) Enzyme-mediated antioxidant systems, including superoxide dismutase, catalase, glutathione peroxidase, metallothionein, thioredoxin(s)/thioredoxin reductase,
glutaredoxin(s), peroxiredoxin(s), nitric oxide synthase oxygenase, eosinophil peroxidase, etc.

2) Small molecules-mediated antioxidant systems, including glutathione, ascorbic acid, α-tocopherol, vitamin A, β-carotene, NADPH, Coenzyme Q-10, urate, flavonoids etc. (Mates, 2000)

Enzymic antioxidants:

Superoxide dismutase:  
Superoxide dismutase (SOD) destroys the radical superoxide by converting it to molecular oxygen and hydrogen peroxide that can, in turn, be destroyed by catalase or glutathione peroxidase reactions.

\[ O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \]

There are two major types of SOD, CuZn-SOD and Mn-SOD, with copper and zinc or manganese ions at the active sites, respectively. Moreover, the enzymes are structurally different. Mn-SOD has a molecular weight of 40,000 and CuZn-SOD 32,000 dalton. Despite different structures, the two enzymes catalyse the same reaction. CuZn-SOD is mainly found in the cytosol of the cell but also to a lesser extent in the lysosomes and the nucleus. Mn-SOD is considered to be a mitochondrial enzyme only. Limited SOD activity is found extracellularly, the majority of this activity is due to a special extracellular SOD (EC-SOD) which contains Cu and Zn but is otherwise structurally different from the intracellular CuZn-SOD (Marklund, 1982).

Catalase:  
Catalase is a tetrameric heme-enzyme consisting of four identical, tetrahedrally-arranged subunits. Therefore, it contains four ferriprotoporphyrin groups per molecule. Catalase is one of the most efficient enzymes known and cannot be saturated by hydrogen peroxide at any concentrations (Lledias et al., 1998). Catalase reacts with H₂O₂ to form water and molecular oxygen, and with H⁺ donors (such as methanol, ethanol, formic acid, phenol etc.) using 1 mole of peroxide in a type of peroxidase activity.

\[ 2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2 \]

\[ \text{ROOH} + \text{AH}_2 \rightarrow \text{H}_2\text{O} + \text{ROH} + \text{A} \]
Catalase protects from $\text{H}_2\text{O}_2$ generated in the cells and even though catalase is not essential for some cell types under normal conditions, it plays a role in the acquisition of tolerance to oxidative stress and the adaptive response of cells (Hunt et al., 1998).

Glutathione peroxidase/reductase system (GPx/GRD):
This system consists of several components including the enzymes glutathione peroxidase and glutathione reductase and the cofactors glutathione and NADPH. All components together efficiently remove hydrogen peroxide. GPx uses GSH as a substrate, forming oxidized glutathione (GSSG), water and/or an organic alcohol (Chance et al., 1979). GPx exists as four isoforms in human, which contain selenium at their active centre. The first isoenzyme GPxl prevents apoptosis induced by oxidative stress. The second and the third isoenzymes are found in the gastrointestinal tract and in plasma, respectively. The fourth form acts directly on membrane phospholipids hydroperoxides and detoxifies them.

$$\text{GPx}$$
$$2 \text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GSSG} + 2 \text{H}_2\text{O}$$

$$\text{GPx}$$
$$2\text{GSH} + \text{R(OOH)COOH} \rightarrow \text{GSSG} + \text{R(OH)COOH} + \text{H}_2\text{O}$$

$$\text{GR}$$
$$\text{GSSG} + \text{NADPH} + \text{H}^+ \rightarrow \text{GSH} + \text{NADP}^+$$

Glutathione reductase stimulates the reduction of GSSG to GSH and maintains glutathione redox status (Deneke and Fanburg, 1989). This ensures a steady supply of the reductive substrate, NADPH to GPx.

Glucose-6-phosphate dehydrogenase (G-6-PDH), ancillary enzyme supports the activity of GPx and GR by supplying reducing equivalents (NADPH) necessary for cellular function and important for the regeneration of oxidized antioxidants; the regeneration of oxidized glutathione, GSSG, to the reduced form, GSH, by reduced nicotinamide dinucleotide (NADH) (Chance et al., 1979).

$$\text{G-6-PDH}$$
$$\text{Glucose-6-Phosphate} + \text{NADP}^+ \rightarrow 6\text{-phosphogluconate} + \text{NADPH}$$
Glutathione-s-transferases:
As similar to glutathione peroxidase, glutathione-s-transferases also reduce peroxides at the expense of GSH. Nevertheless, GST is not involved in the reduction of H$_2$O$_2$.

\[
\text{ROOH + GSH } \rightarrow \text{ GSOH + ROH}
\]
\[
\text{GSOH + GSH } \rightarrow \text{ GSSG + H}_2\text{O}
\]

GST also catalyze the conjugation of electrophile xenobiotics and related intermediates with reduced glutathione (GSH) to produce less toxic derivatives (Morgernstern et al., 1980).

Non-enzymic antioxidants:

Glutathione:
Glutathione is a tripeptide that is composed of L-glutamate, L-Cysteine and glycine. It exists intracellularly in both reduced form (GSH) and an oxidized dimeric form (GSSG) with the reduced/oxidized ration being maintained at around 98:2. The alteration in GSH/GSSG ratio serves as a sensitive marker of oxidative stress (Toborek and Henning, 1994). GSH plays a crucial role in the redox status of the cell (Chance et al., 1979). GSH scavenges singlet oxygen as well as the most reactive hydroxyl radical in an effective manner (Coyle and Puttfarcken, 1993). The ability of GSH in neutralizing the peroxides through GPx and GST reactions render the first line of intra cellular antioxidant defense.

Vitamin C (Ascorbic acid):
Ascorbate acts as an antioxidant by being available for energetically favourable oxidation. Reactive oxygen species such as the hydroxyl radical (formed from hydrogen peroxide), contain an unpaired electron, and thus are highly reactive and damaging to humans and plants at the molecular level. This is due to their interaction with nucleic acid, proteins, and lipids. Reactive oxygen species oxidize ascorbate first to monodehydroascorbate and then dehydroascorbate. The reactive oxygen species are reduced to water, while the oxidized forms of ascorbate are relatively stable and unreactive, and do not cause cellular damage. Ascorbic acid is also able to regenerate $\alpha$-tocopherol at the aqueous-lipid interface (May et al., 1998). After interacting with the $\alpha$-tocopheroxy radical, the oxidized form of ascorbic acid, dehydroascorbic acid, is
produced. Glutathione can re-reduce the oxidized dehydroascorbic acid or interact directly with α-tocopherol to re-reduce that molecule (Leedle and Aust, 1990).

**Vitamin E (Tocopherol):**

α-tocopherol is lipid-soluble and does not provide protection by acting as a sacrificial oxidant. Instead, protection by α-tocopherol is believed to be due to its ability to act as a chain-breaking antioxidant, scavenging peroxyl radicals and thus preventing the propagation of lipid peroxidation processes (Traber and Sies, 1996). It is important to highlight the fact that α-tocopherol is only one form out of the eight naturally-occurring forms of vitamin E, but it has been suggested that α-tocopherol is the most efficient antioxidant among them. However, several studies have shown that various forms of vitamin E have different effects on cell signaling systems (Jackson et al., 2002).

---

**Figure 5: Classification of antioxidant cellular-defense mechanisms.**

(Adapted from: Kohen and Nyska, 2002)
Table 2: Antioxidant enzymes and human diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Specification</th>
<th>Main Key enzyme/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergy</td>
<td>Intolerance of aspirin</td>
<td>GPx</td>
</tr>
<tr>
<td>Allergy</td>
<td>Intolerance to other drugs</td>
<td>SOD</td>
</tr>
<tr>
<td>Allergy</td>
<td>Intolerance to some foods</td>
<td>GPx</td>
</tr>
<tr>
<td>Allergy</td>
<td>Reaction in skin tests</td>
<td>SOD</td>
</tr>
<tr>
<td>Cancer</td>
<td>Bowel</td>
<td>CAT, GPx, SOD</td>
</tr>
<tr>
<td>Cancer</td>
<td>Breast</td>
<td>GPx</td>
</tr>
<tr>
<td>Cancer</td>
<td>Colorectal</td>
<td>COX-2*</td>
</tr>
<tr>
<td>Cancer</td>
<td>Kidney</td>
<td>CAT, GPx, SOD</td>
</tr>
<tr>
<td>Cancer</td>
<td>Leukemia</td>
<td>CAT, GPx, SOD</td>
</tr>
<tr>
<td>Cancer</td>
<td>Liver</td>
<td>CAT, GPx, SOD</td>
</tr>
<tr>
<td>Cancer</td>
<td>Skin</td>
<td>GPx</td>
</tr>
<tr>
<td>Cardiological and vessels injuries</td>
<td>Ischemia</td>
<td>SOD</td>
</tr>
<tr>
<td>Cardiological and vessels injuries</td>
<td>Atherosclerosis</td>
<td>SOD</td>
</tr>
<tr>
<td>Infectious disease</td>
<td>Arthritis</td>
<td>COX-2*</td>
</tr>
<tr>
<td>Infectious disease</td>
<td>Helicobacter pylori</td>
<td>SOD</td>
</tr>
<tr>
<td>Infectious disease</td>
<td>Hepatitis</td>
<td>GPx</td>
</tr>
<tr>
<td>Infectious disease</td>
<td>HIV</td>
<td>GPx</td>
</tr>
<tr>
<td>Infectious disease</td>
<td>Influenza virus</td>
<td>CAT, GPx, SOD</td>
</tr>
<tr>
<td>Genetic disorder</td>
<td>Chronic granulomatous disease</td>
<td>CAT</td>
</tr>
<tr>
<td>Genetic disorder</td>
<td>Down's syndrome</td>
<td>SOD</td>
</tr>
<tr>
<td>Metabolic malfunction</td>
<td>Diabetes</td>
<td>CAT, SOD</td>
</tr>
<tr>
<td>Neurodegenerative disease</td>
<td>Allergic encephalomyelitis</td>
<td>NOS*</td>
</tr>
<tr>
<td>Neurodegenerative disease</td>
<td>Alzheimer's disease</td>
<td>SOD</td>
</tr>
<tr>
<td>Neurodegenerative disease</td>
<td>Amyotrophic lateral sclerosis</td>
<td>SOD</td>
</tr>
<tr>
<td>Neurodegenerative disease</td>
<td>Huntington's disease</td>
<td>SOD</td>
</tr>
<tr>
<td>Neurodegenerative disease</td>
<td>Parkinson's disease</td>
<td>GPx</td>
</tr>
<tr>
<td>Neurodegenerative disease</td>
<td>Prion disease</td>
<td>SOD</td>
</tr>
<tr>
<td>Ophthalmologic problem</td>
<td>Cataract</td>
<td>CAT, SOD</td>
</tr>
</tbody>
</table>

*COX-2: cyclooxygenase-2, *NOS: Nitric oxide synthase

(Source: Matés and Sánchez-Jiménez. 1999)
It is important that sufficient amounts of α-tocopherol, reduced ascorbic acid and reduced glutathione be present within the cell so as to provide protection against oxidative injury.

**Plants as antimicrobial and antioxidant agents:**

Bacterial infection has been found to increase the formation of reactive oxygen species (ROS) (Sakaguchi and Furusawa, 2006; Victor et al., 2005). In addition, bacterial infection often results in increased interleukin secretion, and elevated oxidative stress and/or altered fibronectin levels in infected animals or humans (Alexander and Hudson, 2001; Yao et al., 1995; Yoh et al., 2000; Bost et al., 2000; Kastenbauer et al., 2002). Plant extracts may protect against infections by stimulating or protecting the immune system of the user. Immune system stimulation is associated with antioxidant activity. Plants with multiple protective mechanisms as antibacterial and antioxidant agents may be one way of minimizing infections and tissue injury due to oxidative stress.

et al., 2008), *Tamarix ramosissima* (Sultanova et al., 2001), *Rhaphidophora pertusa* (Sasikumar and Doss, 2006), *Hippophae rhamnoides* L. (Negi et al., 2005), *Rumex japonicus* (Elzaawely et al., 2005), *Pericarpium Citri Reticulatae* (ZhiBiao Yi et al., 2008) and *Rosmarinus officinalis* (Moreno et al., 2006).

The clinical efficacies of many plant preparations used are not yet validated (Ali et al., 2008). Therefore if a systematic investigation is initiated in the traditional medicinal systems practised in India, it can offer promising leads for the discovery of potent antimicrobials and antioxidants that can have therapeutic use globally.

Plants selected in the study:

*Actiniopteris radiata* (Sw.) Link. (Actiniopteridaceae), *Corallocarpus epigaeus* (Rottl. and Willd.) Clarke. (Cucurbitaceae) and *Senecio tenuifolius* Burm. f. (Asteraceae) are the plants selected in the present study, according to the ethnobotanical information which possess antimicrobial and antioxidant activities. Till date, there is no scientific evidence regarding the anti-*Staphylococcus aureus* and antioxidant activities of the above selected plants. In the present study, the selected plants were first assessed for their *in vitro* efficacy of anti-*Staphylococcus aureus* and antioxidant activities, to short list a potent plant for further *in vivo* antioxidant activity by employing CCl₄ as oxidative stress inducer.
OBJECTIVES AND ORGANISATION OF THE THESIS
OBJECTIVES AND ORGANISATION OF THE THESIS:

Modern medicines have little to offer for alleviation of degenerative diseases and it is chiefly the plant-based preparations, which are employed for their treatment of liver disorders. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine. The main aim of the present study is to investigate and identify a potent plant extract among the selected plants (*Actiniopteris radiata*, *Corallocarpus epigaeus*, and *Senecio tenuifolius*) for anti-*Staphylococcus aureus* and antioxidant activities. The objectives of the study are as follows.

- Preparation of the plant extracts in the order of their increasing polarity for anti-*Staphylococcus aureus* and antioxidant activity.
- *In vitro* evaluation of anti-*Staphylococcus aureus* and antioxidant activity of the prepared plant extracts.
- Potent plant selection, based on *in vitro* activity and phytochemical screening.
- Evaluation of the toxicity of the selected plant extract.
- Evaluation of *in vivo* antioxidant activity of against carbon tetrachloride induced toxicity in rats.

The thesis is organized in the sequence of Introduction followed by Chapter-1 which deals with *in vitro* anti-*Staphylococcus aureus* and antioxidant activities, Chapter-2 examines the safety evaluation of ethanol extract of *A. radiata* and Chapter-3 describes the *in vivo* antioxidant activity of *A. radiata*. Summary and Conclusions were provided separately along with the Future Directions. Bibliography is listed at the end of the thesis.