Chapter 1

GENERAL INTRODUCTION

1.1 Global Burden of Bacterial and Fungal Infection

Efforts to reduce disease on Earth in human beings have not yet been very successful. The worldwide death toll is 52 million people a year according to the estimate by WHO (World Health Organization), and a third of them 17 million people die from infectious disease (Junko, 2006). Some commonly encountered pathogens have been associated with some of the human diseases (Okigbo et al., 2005). The awareness of microbial diseases, availability of better diagnostic tools and discovery of improved therapeutic agents have stimulated a great deal of scientific activity in the area of Medical Microbiology. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. The majority of clinically used antimicrobial drugs have drawbacks in terms of toxicity, efficacy, cost and their frequent use has led to the emergence of resistant strains. Thus, there is an urgent need to develop alternative biodegradable agents, which would be free from side effects. This search prompted the exploration of Natural Algal products, that could be exploited as biodegradable, more systemic and non-toxic antimicrobial agents, which would be free from side effects and with microbial toxic properties. In recent years, there has been growing interest in alternative therapies and the use of algal products, for antimicrobial resistance. The algal kingdom is a vast repertoire of phycochemicals and constitutes a promising area of current research in phycochemical prevention. It is therefore reasonable to anticipate a huge reservoir of biomolecules of algae, which can be therapeutic agents.
So far more than half of the flora has been documented for pharmacological importance; still the vast majority has not yet been scientifically evaluated. For an eco-friendly environment, the use of phycochemicals is now emerging as one of the prime means for the control of diseases particularly in countries, which are rich in algal biodiversity. Advancement made in the application of chromatographic methods for isolation and various spectroscopic techniques for structural elucidation have strengthened the research base in the field of phyco-chemistry. Need for discovery of new antimicrobial agents active towards the resistant strains of paramount importance. Since, the most infectious diseases in microbiological origin with the advent of ever increasing resistant fungal and bacterial strains, there has been a corresponding rise in the universal demand for natural antimicrobial therapeutics. Fungi and bacteria cause important human diseases, especially in tropical regions and in immunocompromised or immunodeficient patients. Despite the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs (Silver and Bostain, 1993).

Therefore, to explore the possibility of native algae of nearby areas for their bioefficacies, in order to reduce the risk of microbial disease in human and animals followed by chemical characterization of their bioactive principles is the need of the day.
1.2 **Bacterial Infection**

We are constantly in contact with a myriad of micro-organisms in the environment. However, we are in even more intimate contact with an enormous number of micro-organisms that inhabit our bodies. There are thousands of species of bacteria which cause variety of disease in human beings. In recent years infections caused by bacteria resistant to multiple antibiotics have been a significant problem. Some commonly encountered pathogens have been associated with some of the human diseases. Methicillin resistant *Staphylococcus aureus* (MRSA) has been troubling hospital services all over the world (Archibald *et al.*, 1997; Smith *et al.*, 1999).

*Staphylococcus aureus* (literally “Golden Cluster Seed”) is the most common cause of Staph infection, is spherical bacterium, frequently living on the skin or in the nose of a person, that can cause a range of illness from minor skin infections, such as pimples, impetigo, boils, cellulites and abscesses, to life-threatening diseases, such as pneumonia, meningitis, endocarditic, and septicemia. *Staphylococcus aureus* is a facultatively anaerobic, gram positive bacteria, which causes food poisoning and usually grow on the nasal membrane and skin. It is also found in the gastrointestinal and urinary tracts of warm blooded animals. *S. aureus* can cause life-threatening diseases such as, meningitis, osteomyelitis, endocarditis, bacteremia, and sepsis. Its incidence ranges from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections and is often the cause of
postsurgical wound infections. Every year, about 500,000 patients in American hospitals contact a *Staphylococcal* infection. It causes boils, abscesses wound infection, pneumonia, toxic shock syndrome and other diseases (Cheesbrough, 2000). Also the worldwide emergence of *E. coli* and many other β-lactamase producers became a major therapeutic problem (Ferreira *et al.*, 2004).

*E.coli* is a gram negative bacteria usually motile is an extremely versatile opportunistic pathogen (Cheesbrough, 2000) causes septic mias and can infect the gall bladder, meninger, surgical wound, skin lesions, and the lungs especially in debilitate and immuno-deficient patients (Black, 2000). *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 flucloxacllin are able to resist degradation by staphylococcal penicillinase. Vancomycin-resistant *S.aureus* (VSRA) is a strain that become resistant to the glycopeptides. The first case of Vancomycin-
intermediate *S. aureus* was reported in Japan.

![Image of human with labeled infections]

**Figure 1**: Sites of *Staphylococcal* infections

Another highly infectious microbe *Salmonella typhimurium* also causes serious diseases. *Salmonella* genus of rod-shaped, Gram-negative, non-spore-forming, predominantly motile enterobacteria. The genus *Salmonella* was ultimately named after Daniel Elmer Salmon, an American veterinary pathologist. *Salmonella* infections are zoonotic and can be transferred between humans and non-human animals. Many infections are due to ingestion of contaminated food. *Salmonella* infection may spread from the intestines to the blood stream, and then to other body sites, and can cause death unless the person is treated promptly with antibiotics. The elderly, infants, and those with impaired immune systems are more likely to develop severe illness.
It is estimated that every fifth person in Germany is a carrier of *Salmonella*. In the USA, there are approximately 40,000 cases of *Salmonella* infection reported each year. According to the World Health Organization, over 16 million people worldwide are infected with typhoid fever each year, with 500,000 to 600,000 fatal cases.

### 1.3 Fungal Infection

Fungi are ubiquitous in the environment and unavoidable. Spores of fungi are found everywhere in the environment, floating in air, which may land on skin or inhaled causing wide range of skin infections (Mycoses). This large and diverse kingdom comprises more than 100,000 recognized species. Of this large group, only about 300 species have been identified as human pathogens; however, more than three fourths of these pathogens infect primarily the skin or subcutaneous tissues. There has been a dramatic increase in the incidence of fungal infections since 1980’s (Mackenzie *et al*., 1986; Haria and Bryson, 1995; Laube, 2004).

#### 1.3.1 Dermatomycosis

Dermatomycosis includes dermatophytosis, sporotrichosis and cryptococcosis commonly occurring in humans and animals and are considered to be zoonotic diseases. The dermatophytosis is classified in three anamorphic (asexual or imperfect) genera, *Epidermophyton*, *Microsporum*, and *Trichophyton*, of anamorphic class Hyphomycetes of the Deuteromycota (Fungi imperfecti). Dermatophytosis is a clinical condition caused by fungal infection of the skin in humans, pets and domestic animals. It is caused by fungi of several different species. The fungi that causes parasitic infection (Dermatophytes) feed on keratin, the material found in the outer layer of the
skin, hair and nails. These fungi thrive on skin that is warm and moist but may also survive directly on the outsides of hair shafts or in their interiors.

This condition has been prevalent since before 1906, at which time ringworm was treated with compounds of mercury, or sometimes sulphur or iodine. Hairy areas of skin were considered too difficult to treat, so the scalp was treated with X-rays and followed up with anti-parasitic medication. It has been estimated that in current times, up to twenty percent of the population is infected by ringworm or one of the other dermatophytoses.

### 1.3.2 Dermatophytes

Dermatophytes (name based on the Greek for ‘skin plants’) are a common label for a group of three types of fungus that commonly causes skin diseases in animals and humans. These anamorphic (asexual or imperfect fungi) genera are: *Microsporum*, *Epidermophyton* and *Trichophyton*. There are about 40 species in these three genera which are classified on the basis of the infection site. The classification of dermatophytes shown in (Table-1)

Dermatophytes prefer to grow at low temperature up to 30°C. Higher temperature of the hot spot cause the fungus to move towards the periphery of the lesion where the temperature is comparatively low, thus producing concentric ring shaped lesions; the name “ring worm diseases” is derived from such lesions.
<table>
<thead>
<tr>
<th>Dermatophytosis</th>
<th>Affected Body Parts</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tinea pedis</em> (Athlete’s foot)</td>
<td>Feet</td>
</tr>
<tr>
<td><em>Tinea unguium</em></td>
<td>Finger nails and toenails</td>
</tr>
<tr>
<td><em>Tinea corporis</em></td>
<td>Arms, legs and trunk</td>
</tr>
<tr>
<td><em>Tinea cruris</em> (Jock itch)</td>
<td>Groin area</td>
</tr>
<tr>
<td><em>Tinea manuum</em></td>
<td>Hands and palm area</td>
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<tr>
<td><em>Tinea capitis</em></td>
<td>Scalp</td>
</tr>
<tr>
<td><em>Tinea barbae</em></td>
<td>Facial hair</td>
</tr>
<tr>
<td><em>Tinea faciei</em> (Face fungus)</td>
<td>Face</td>
</tr>
<tr>
<td>Other superficial mycoses</td>
<td>Caused by</td>
</tr>
<tr>
<td><em>Tinea versicolor</em></td>
<td><em>Malassezia furfur</em></td>
</tr>
<tr>
<td><em>Tinea nigra</em></td>
<td><em>Hortaea werneckii</em></td>
</tr>
</tbody>
</table>

**Table-1: Body parts affected by Dermatophytosis**

Tinea corporis

Tinea capitis
Dermatophytes colonize the superficial dead and desquamating layers of skin and appendages and cause infections of the skin, hair and nails due to their ability to obtain nutrients from keratinized material. The organisms colonize the keratin tissues and inflammation is caused by the host response to metabolic by-products. They are usually restricted to the non living cornified layers of the epidermis because of their inability to penetrate viable tissue of an immune-competent host. Invasion does elicit a host response ranging from mild to severe. Acid proteinases, elastase, keratinase and other proteinases reportedly act as virulence factor.

The development of cell mediated immunity correlated with delayed hypersensitivity and an inflammatory response is associated with clinical culture, whereas the lack of or a defective cell-mediated immunity predisposes the host to chronic or recurrent dermatophytic infection. Some of these infections are known as ringworm or tinea. Toenail or fingernail infections are referred to as Onychomycoses.

### 1.3.3 Candidosis

In recent years, candidosis has been suggested as the preferred term to describe infections that occur as the result of mucocutaneous or systemic infection by *Candida* organisms, replacing the terms candidiasis and moniliasis. Although other species may produce disease, particularly in immuno-compromised individuals, *Candida albicans* is the most common pathogen. *Candida* species may become opportunistic pathogens under a variety of circumstances including pre-existing dermatitis, maceration, diabetes mellitus, antibiotic therapy or immune-suppression. Unlike the dermatophytes, *Candida* sp. organisms do not utilize keratin as a substrate for growth but prefer areas with high concentrations of serum or glucose (Table 2)
Table 2: Pathogenic Candida species

*C. albicans* prefers high humidity and a damaged stratum corneum for growth. Candidosis is more likely to be painful than dermatophytosis, presumably due to invasion of viable tissue and a brisk host response (Dreizen, 1984). Candidosis is the most common fungal infection of the oral mucosa (Rebora et al., 1973). Oral candidosis most commonly affects newborns, the elderly and patients who have diabetes or are immunocompromised. It is mentioned that above two decades ago *C. albicans* was commonly regarded as little more than culture contaminant, however, because of developed resistance, in recent years this organism has become a major human
pathogen (Michael and Pharm, 2001). Frequency of *C. albicans* infections has risen dramatically and the development of drug resistant *C. albicans* is a major concern worldwide (Goff *et al.*, 1995 and Nolte *et al.*, 1997).

1.4 **Antibiotics: Types & Roles**

Antibiotic is a chemical substance produced by a micro organism that inhibits the growth of or kills other micro organisms. The term “antibiotic” was first used in 1942 by Dr. Selman A. Waksman, soil microbiologist. Dr. Waksman and his colleagues *discovered several actinomycetes derived antibiotics*. Antibiotics that kill bacteria are called "bactericidal". Antibiotics that stop the growth of bacteria are called "bacteriostatic". An antibiotic in a broader sense is a chemotherapeutic agent that inhibits or abolishes the growth of microorganisms, such as bacteria, fungi, or protozoa. Other terms which are often used are chemotherapeutics or antimicrobials, however, these terms are not synonymous. For example, antimicrobials can also be effective against viruses. The expression “chemotherapeutical” refers to compounds used for the treatment of disease which kill cells, specifically microorganisms or cancer cells. In popular usage, it often refers to anti-neoplastic drugs used to treat cancer. The term antibiotic originally referred to any agent with biological activity against living organisms; however, “antibiotic” now refers to substances with antibacterial, anti-fungal, or anti-parasitical activity. There are currently about 250 different chemical entities registered for use in medicine and veterinary medicine.

1.4.1 **Antifungal drugs**
Fungal infections are caused by eukaryotic organism and for that reason they generally present more difficult therapeutic problems than other infections. There are relatively few agents that can be used to treat fungal infections. The fungal cell wall may be considered to be a prime target for selectively toxic antifungal agents because of its chitin structure, absent from human cells. No clinically available inhibitor of chitin synthesis analogous to the beta-lactoms exists at present; even through much effort is being directed towards developing such agents (Arikan et al., 1999 and Boucher et al., 2004). Other targets are currently being exploited. Last 25 years have been seen the discovery of number of synthetic antifungal compounds, which are available for both oral and sometimes topical use.

The following classes of antifungal antibiotics are established in the market:

**Polyene antibiotics:**

Polyene antibiotics (Nystatin and Amphotericin B) binds to sterols within the fungal membrane, disrupting its integrity. This makes the membrane leaky, leading to loss of small molecules from the fungal cell (Goodwin et al., 1995; Williams, 1999). Amphotericin B is produced by *Streptomyces nodosus* and nystatin is produced by *Streptomyces noursi*. These antibiotics show activity against major systemic mycosis, including coccidiomycosis, blastomycosis, sprotrichosis, cryptococciosis and candidosis.

**Azole antibiotics:**

Imidazole and Triazoles: It is the emerging group of antifungal agents that act to inhibit synthesis of ergosterol by blocking the action of 14-alpha-demethylase, a component of fungal membrane. These drugs, like the polyene antibiotics, may cause leakage of
small molecules out of fungal cells. They have broad spectrum antifungal activity although there is some variation of activity between the various compounds (Lewis et al., 1984; Lyman and Walsh, 1992; Debruyne and Ryckelynck, 1993; Sheehan et al., 1999 and Ullmann, 2003).

Allylamines antibiotics:

These agents act by inhibiting squalene epoxidase. This is another enzyme in the pathway that leads to synthesis of ergosterol, so these agents are conceptually related to the azole antifungal agents (Syed and Maibach, 2000).

Glucan synthesis inhibitors (Echinocandin antibiotics):

a. The echinocandin lipopeptide group: Echinocandin B (ECB) is a cyclic lipopeptide with antifungal activity. It was isolated by (Benz et al., 1974) from Aspergillus nidulans var. echinulatus. A new class of antifungal drug, the echinocandin has recently appeared on the market and launched for invasive aspergillosis (Ponikau et al., 2002). Drug interaction issues are a major impediment to the use of the azole antifungals (Stevans et al., 2000). The echinocandin lipopeptide have potent anticandidiasis activity. Although ECB causes red blood cell lysis.

b. The papulacandin group: Papulacandins are group of naturally occurring glycolipid antifungal agent which resembles ECB in their mode of action and activity. This group includes papulacandin and chaetiacandin (Aoki et al., 1993). The glucan synthesis inhibitors block fungal cell wall synthesis by inhibiting the enzyme 1,3 β-glucan synthase (Arathoon et al., 2002).
**Chitin synthesis inhibitors:**
Nikkomycin and polyoxins are nucleoside di and tri peptide respectively. These are potent inhibitors of chitin synthesis, which is responsible for chitin synthase, present in the fungal cell wall. These antibiotics are competitive inhibitors has been found to show broader antimicrobial spectrum. Enhanced activity has been observed against *C. albicans* when a combination of nikkomycin and azoles were used *in vivo* (Hector and Schalter, 1991).

**Mannan antifungal antibiotics:**
The pradimicins and benanomycin, a unique group of antifungal antibiotics, were isolated from actinomycetes, *Actinomadura hibisca* (Oki *et al.*, 1990). These are highly colored benzo- nepthaquinone derivatives (Gomi *et al.*, 1988).

**Other topical agents:**
There are a number of topical agents used in treatment of superficial cutaneous mycoses, oropharyngeal candidiasis. The superficial cutaneous mycoses that respond to topical therapy include the localized infection of hair, nails and epidermis due to the dermatophyte and *Candida* (Hart *et al.*, 1999).

**1.4.2 Antibacterial drugs**
The assessment of the activity of an antibiotic is crucial to the successful outcome of antimicrobial therapy. Non-microbiological factors such as host defense mechanisms, location of an infection, underlying disease as well as the intrinsic pharmacokinetic and pharmacodynamic properties of the antibiotic (Pankey and Sabath, 2004), fundamentally, antibiotics are classified as either having lethal or bactericidal action.
against bacteria or are bacteriostatic, preventing bacterial growth. The bactericidal activity of antibiotics may be growth phase dependent and in most but not in all cases the action of many bactericidal antibiotics requires ongoing cell activity and cell division for the drugs killing activity (Mascio et al., 2007). The activity of antibiotics may be concentration-dependent and their characteristic antimicrobial activity increases with progressively higher antibiotic concentrations. They may also be time dependent, where their antimicrobial activity does not increase with increasing antibiotic concentrations; however, it is critical that a minimum inhibitory concentration is mentioned for a certain length of time (Rhee and Gardiner, 2004).

Antibacterial drugs are commonly classified based on their mechanism of action, chemical structure or spectrum of activity. Most antibiotics target bacterial functions or growth processes (Calderon and Sabundayo, 2007). Antibiotics which target the bacterial cell wall (penicillins, cephalosporins), or cell membrane (polymixins), or interfere with essential bacterial enzymes [quinolones, sulfonamides] usually are bactericidal in nature.

Those which target protein synthesis, such as the amino-glycosides, macrolides and tetracyclines, are usually bacteriostatic (Finberg et al., 2004). Further categorization is based on their target specificity: “narrow spectrum” antibiotics target particular types of bacteria, such as gram-negative or gram-positive bacteria, while broad-spectrum antibiotics affect a wide range of bacteria. In the last few years three new classes of antibiotics have been brought into clinical use.

1.5 Biologically Active Compounds from Cyanobacteria
Cyanobacteria produce a wide variety of chemically unique substances derived from secondary biosynthesis. These compounds are called secondary metabolites because they are not used by the organism for its primary metabolism, such as cell division, photosynthesis or respiration. It was suggested that the secondary metabolites are produced for protection against grazers, to control the growth of bacteria and green algae, as storage forms, or as detoxification product.

Systematic screening of cyanobacteria for bioactivity (Patterson et al., 1991; 1993; 1994; Falch et al., 1995; Jaki et al., 2000) showed that these microorganisms are a rich source of novel bioactive agents. The discovery rate for bioactive compounds is about 7%, about the same as that found for other microorganisms. The rate of rediscovery of known bioactive compounds is, however; significantly lower among the cyanobacteria than among Actinomycetes. Thus, cyanobacteria have a good potential for providing pharmaceutical substances with unusual or unknown chemical structures.

1.6 Cyanobacterial toxins, their source, chemical nature

Toxin
Most toxins are classified as hepatotoxins, neurotoxins or dermatotoxins after the symptoms they produce. However, because those symptoms have been mainly described in vertebrates, in the context of this review it is more relevant to classify them according to their chemical structures [cyclic peptides, alkaloids, lipopolysaccharides and polyunsaturated fatty acids (PUFAs) and their derivatives]. Some recent studies have focused on the effects of toxins on plankton and
macrophages. The effects of the toxin may be direct or indirect, linked to the metabolism of the molecule by the detoxification system.

**Cyclic peptides:** Two toxins are cyclic peptides: Microcystins and Nodularins. Microcystins being the most widely distributed toxins. Microcystins are produced by planktonic cyanobacteria belonging to the genera *Anabaena, Microcystis, Planktothrix* and by some species of the benthic *Oscillatoria*. Nodularins are produced by *Nodularia spumigena* (Briand *et al.*, 2003). These peptides contain unusual amino acids and show a strong structural variability: more than 75 structural variants of microcystin have been described to date. Microcystins and nodularins have been shown to be inhibitors of the serine/threonine protein phosphatases types 1 and 2A (Mac Kintosh *et al.*, 1990). This activity has been demonstrated for mammals and higher plant protein phosphatases. The toxin–enzyme interactions are very strong, and binding is essentially stoichiometric. The concentration required to inhibit protein phosphatases *in vitro* is lower for nodularin than for microcystins (Ohta *et al.*, 1994). Inhibition of protein phosphatases leads to hyperphosphorylation of proteins associated with the cytoskeleton and consequent redistribution of these proteins. In mammals and birds, the toxic effects of microcystins are almost restricted to the liver.

Besides these well-studied effects of microcystins against vertebrates, several cases of negative effects of these hepatotoxins against micro-algae or aquatic plants have been reported. The oxidative stress induced in the dinoflagellate *Peridinium gatunense* by microcystin-LR is linked to the activation of mitogenactivated protein kinases, enzymes known to play a role in cellular responses to biotic and abiotic signals in mammals and higher plants cells (Vardi *et al.*, 1999; 2002).
**Alkaloids:** The alkaloid toxins include anatoxin-a (and homoanatoxin-a), anatoxin-a (s), cylindrospermopsins and saxitoxins. Anatoxins are mainly produced by Anabaena species, but also by *Microcystis* and *Oscillatoria* (Park *et al.*, 1993; Sivonen and Jones, 1999). Cylindrospermopsins are produced by *Aphanizomenon ovalisporum, Cylindrospermopsis raciborskii, Raphidiopsis curvata* and *Umezakia natans* (Briand *et al.*, 2003).

**Saxitoxins:** were first described in marine dinoflagellates but they have been recently identified in five freshwater cyanobacterial species: *Aphanizomenon flosaquae, Anabaena circinalis, Cylindrospermopsis raciborskii, Lyngbia wollei* and *Planktothrix* sp. Anatoxin-a and anatoxin-a (s), two unrelated compounds, both inhibit transmission at the neuromuscular junction.

**Anatoxin-a:** is a cholinergic aganist that binds to nicotinic acetylcholine receptor while anatoxin-a(s) is an acetylcholinesterase inhibitor with a mechanism similar to that of organo-phosphorus insecticides. Toxic effects observed on the aquatic plant *Lemna minor* may be linked to the metabolism of the toxin by the plant that may produce either reactive species of oxygen or a new compound toxic for the plant (Mitrovic *et al.*, 2004). To date there is no known effect of the saxitoxins on aquatic plants or on micro-algae.

**Polyunsaturated fatty acids and their derivatives:**

Compounds containing the unsaturated aldehyde structure (2,4-heptadienal, 2,4-octadienal) from diatoms act against herbivores. Cell division is blocked by the aldehydes, certainly because of microtubule de-polymerisation whereby tubulin cannot
organise into filaments (Buttino et al., 1999). Moreover, no DNA replication can occur in presence of the aldehyde. In copepods, the polyunsaturated aldehyde induces a caspase-independent programmed cell death as revealed by cytochemical and biochemical approaches (Romano et al., 2003). In sea urchin, the aldehyde (2E,4E-decadienal) induces apoptosis and activates a caspase 3-like protease.

Table 3: Toxins: Their source, chemical nature and effects

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Source</th>
<th>Chemical nature</th>
<th>Effect</th>
</tr>
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<tbody>
<tr>
<td>Neurotoxin</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Anatoxin-a</td>
<td>Anabaena, Aphanizomenon, Cylindrospermum, Oscillatoria, Phormidium, Rhaphidiopsis</td>
<td>Alkaloid</td>
<td>Binds irreversibly to the nicotinic acetylcholine receptors</td>
</tr>
<tr>
<td>Anatoxin-a (s)</td>
<td>Anabaena, Aphanizomenon, Anabaena, Cylindrospermopsis, Lyngbya, Planktothrix</td>
<td>Guanidine methyl phosphate ester</td>
<td>Inhibits acetylcholinesterase</td>
</tr>
<tr>
<td>Saxitoxins</td>
<td>Anabaena, Aphanizomenon, Anabaena, Cylindrospermopsis, Lyngbya, Planktothrix</td>
<td>Carbamate alkaloid</td>
<td>Binds and blocks the sodium channels in neural cells</td>
</tr>
<tr>
<td>Hepatotoxins</td>
<td>Anabaena, Anabaenopsis, Hapalosiphon, Microcystis, Nostoc, Oscillatoria, Planktothrix</td>
<td>Cyclic heptapeptide</td>
<td>Inhibition of protein phosphatases</td>
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<tr>
<td>Microcystins</td>
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<tr>
<td>Cytotoxins</td>
<td>Nodularin</td>
<td>Nodularia</td>
<td>Cyclic pentapeptide</td>
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<td>Cylindrospermopsis</td>
<td>Anabaena,</td>
<td>Anabaena,</td>
<td>Guanidine alkaloid</td>
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<td></td>
<td>Aphanizomenon</td>
<td>Cylindrospermopsis,</td>
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<td>Raphidiopsis</td>
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<td>Dermatotoxins</td>
<td>Lyngbyatoxin-a</td>
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<td>Aplysiatoxins</td>
<td>Lyngbya, Oscillatoria,</td>
<td>Lyngbya, Oscillatoria,</td>
<td>Alkaloid</td>
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<td></td>
<td></td>
<td>Schizothrix</td>
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</tr>
<tr>
<td>Endotoxins</td>
<td>Lipopolysaccharide</td>
<td>All cyanobacteria</td>
<td>Lipopolysaccharide</td>
</tr>
</tbody>
</table>

### 1.7 Resistances to antimicrobial drugs

#### A Global Problem

Microbial resistance is a major drawback in chemotherapy of microbial or fungal infection disease. The tissue of resistance to antimicrobial drugs was discussed in annals 10 years ago (Kunin, 1983) as a follow up to the report by the WHO scientific working group on antimicrobial resistance. The emergence of antibiotic resistance is an
evolutionary process that is based on selection for organisms that have enhanced ability to survive doses of antibiotics that would have previously been lethal (Cowen, 2008). The clinical consequences of antifungal resistance are evident in treatment failures as well as in the prevalence of fungi, such as for *Candida* spp. and emerging moulds, causing disease (Baddlev and Pappas, 2005; Nucci and Marr, 2005). Antifungal drug development has lagged far behind that of antibacterial agents. Fungi are eukaryotes and despite the presence of a cell wall, fungi are more similar to mammalian cells on a cellular level than to bacteria. Fungi replicate more slowly than bacteria and are often difficult to quantify, which complicates efficacy assessments (Nosanchuk, 2006).

Resistant bacterial strains have emerged and have spread throughout the world because of the remarkable genetic plasticity of the micro-organisms, heavy selective pressures of use, and the mobility of the world population (Calvin and Kunin, 1993). New and more expensive drugs have appeared almost in the nick of time, but it is doubtful that they will keep pace. The problem of resistance to antibacterial drugs is particularly troublesome in developing countries. The underlying problems are largely economic and societal, and no ready solutions are available. An urgent need exists for more appropriate selection and use of antimicrobial drugs in the developed as well as in developing countries. Variable levels of resistance of micro-organisms to a wide range of antimicrobial agents, including disinfectants and preservatives, have been reported in the scientific literature (Potenski *et al.*, 2003; Kramer *et al.*, 2006; Capita, 2007 and Plumridge *et al.*, 2008). Although much attention is focused on resistance patterns of eubacteria (Tenover, 2006), resistance is being found for virtually all microbial agents including mycobacteria (Andini and Nash, 2006), Viruses (Kuritzkes, 2006 and Monto *et
al., 2006), parasites (Schunk et al., 2006 and Xiao et al., 2006) and fungi (Katiyar et al., 2006 and Mentel et al., 2006).

The discovery of antimicrobial agents by Paul Enrlich was one of the most remarkable discoveries that changed the face of medicinal practice (Wood and Morellering, 2003). The use of algal extract and phycochemical, both with known antimicrobial properties, can be of great significance in therapeutic treatments. Therefore, the search for new antimicrobial drugs among algae became an important alternative as part of our investigation on “Studies on antimicrobial activity of Spirulina platensis (Geitler) and its structural characterization”.
**Algae Cyanobacteria, Spirulina platensis Geitler under Study**

Scientific classification

Domain: Bacteria

Phylum: Cyanobacteria = Chroobacteria

Order: Oscillatoriales

Family: Phormidiaceae

Genus: *Spirulina*

Species: *platensis*

*Spirulina* is the common name for human and animal food supplements produced primarily from two species of Cyanobacteria: *Spirulina platensis*, and *Spirulina maxima*. These and other Arthrospira species were once classified in the genus Spirulina. There is now agreement that they are a distinct genus, and that the food species belong to Arthrospira; nonetheless, the older term *Spirulina* remains the popular name. [Plate 1]

**Biology**

*Spirulina* are free-floating filamentous cyanobacteria characterized by cylindrical, multicellular trichomes in an open left-hand helix. *Spirulina* occurs naturally in tropical and subtropical lakes with high pH and high concentrations of carbonate and bicarbonate. *S. platensis* occurs in Africa, Asia and South America, whereas A. maxima are confined to Central America.

**Nutrients and other chemicals**
**Protein:** It contains an unusually high amount of protein, between 55% and 77% by dry weight, depending upon the source. It is a complete protein, containing all essential amino acids, though with reduced amounts of methionine, cysteine, and lysine when compared to the proteins of meat, eggs, and milk. It is, however, superior to typical plant protein, such as that from legumes.

**Essential fatty acids:** It is rich in gamma-linolenic acid (GLA), and also provides alphalinolenic acid (ALA), linoleic acid (LA), stearidonic acid (SDA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA).

**Minerals:** It is a rich source of potassium, and also contains calcium, chromium, copper, iron, magnesium, manganese, phosphorus, selenium, sodium, and zinc.

**Photosynthetic pigments:** It contains many pigments including chlorophyll-a, xanthophyll, beta-carotene, echinenone, myxoxanthophyll, zeaxanthin, canthaxanthin, diatoxanthin, 3'-hydroxyechinenone, beta-cryptoxanthin, oscillaxanthin, plus the phycobiliproteins c-phycocyanin and allophycocyanin.

**Evidence of health and healing effects:** Despite existing research supporting *Spirulina*’s health and healing properties, detractors claim that these are frequently overstated by *Spirulina* advocates.

**In vitro research:** Its extract inhibits HIV replication in human T-cells, peripheral blood mononuclear cells (PBMC), and Langerhans cells.

**Animal research:** It helps prevent heart damage caused by chemotherapy using Doxorubicin, without interfering with its anti-tumor activity. *Spirulina* reduces the severity
of strokes and improves recovery of movement after a stroke; reverses age-related declines in memory and learning; and prevents and treats hay fever.

**Human research:** It is effective for the clinical improvement of melanosis and keratosis due to chronic arsenic poisoning; improves weight-gain and corrects anemia in both HIV-infected and HIV-negative undernourished children; and protects against hay fever.

*Spirulina* is a microscopic freshwater plant, an aquatic microvegetable/organism composed of transparent bubble-thin cells stacked end-to-end forming a helical spiral filament. *Spirulina* is microscopic blue-green algae that exist as a single celled organism turning sunlight into life energy. It is one of the first life forms designed by nature more than 3.6 billion years ago. Spirulina contains billions of years of evolutionary wisdom in its DNA and is an offspring of earth’s first photosynthetic life forms. Under the microscope, *Spirulina* is a blue-green color and has the appearance of a spiral of long thin threads. It is a simple, one-celled form of algae that thrives in warm, alkaline freshwater bodies. The name ‘*Spirulina*’ is derived from the Latin word for helix’ or ‘spiral’; denoting the physical configuration of the organism when it forms swirling, microscopic strands. *Spirulina* is exceedingly adaptable and occurs in a wide variety of environments including fresh water, tropical springs, saltwater and saltpans.