CHAPTER - I
INTRODUCTION

In the recent years scientists have turned towards the higher plants in their quest for antimicrobial substance. Earlier workers believed that antimicrobial substances can be obtained only from microorganisms. With the increasing interest in antibiotics and antifungal substances, plants as a potential source of such substances are receiving considerable attention. The volatile constituents of various higher plants, i.e. their essential oils have also exhibited potent bioregulatory activity, especially against microorganisms (Mahadevan, 1982; French, 1985). According to Sbragia (1975), the green plants, especially those growing in the tropics are reservoirs of various biologically active substances. Towards the end of the twentieth century a lot of work has been done to study the biotoxic activity of plants extracts and several higher plant products have been standardized as potent biotoxicants against different plant pathogenic fungi. But, very little study has been made to ascertain the activity of such plant products against human pathogenic fungi (Dubey and Mishra, 1990; Kishore et al., 1993). The various synthetic products that are used as biotixicants (antimicrobial or pesticidal) are known to possess severe side effects (Shorey and Mckelvey, 1977; Paterson et al., 1980; Singh and Agarwal, 1987; Patel et al., 1997). In contrast,
the plants products, which are consumed by human beings as part of their diets and are already in use in different forms in the Ayurvedic System of medicine since time immemorial, will have minimum or no side effects on the human body (i.e., there will be least mammalian toxicity).

In nature, every plant has its own role to play and it is a known fact that all living organisms ultimately depend on the plant kingdom for their survival and well-being. It has also been observed that many plants and their parts are used by man and animals alike, for the treatment of a variety of ailments. Higher plants possess a wide range of secondary metabolites, which can be exploited for their different biological properties (Wain, 1977; Kubo and Nakinishi, 1979). The volatile oils present in some plants are such secondary metabolites. Research works in this field have also shown that plants do possess certain active principles or compounds, the concentration of which varies with the varying seasons, age of the plant and the plant part used. The active principles are bio-chemical compounds that can be extracted from the plant parts by various methods (Guenther, 1972; Tewari, 1986). The use of plant products as biotoxicants has tremendous benefit from the point of view, that they are biodegradable and has minimum mammalian toxicity. A large variety of plants have been reported to possess antimicrobial properties (Sehgal, 1961; Dahia and Mukharya, 1977; Joshi and Desai, 1988; Joshi et al., 1990, '91;
Onaolapo et al., 1993; Dushyant and Bohra, 1998; Gehlot and Bohra, 1998). These plants grow luxuriantly in their natural habitats.

People in the rural areas widely depend on the medicinal properties of the local plants for curing their day-to-day ailments. Many instances have come to notice, where village medicine men or ‘ojhas’ have laid claim for the treatment or control of a number of diseases, ranging from minor ones like flu and cold to more serious problems like hepatitis and even cancer and AIDS. At present research work is being conducted to study the possibility of the existence of natural anti-HIV agents (Hoang et al., 2002). The efficiency of the treatment of cancer and AIDS will be proved only by time. Nonetheless, research works carried out by scientists have proved time and again, the medicinal properties of plant, viz. Chopra and Chopra, 1956; Chopra et al., 1956; Jain, 1968; Kritikar and Basu, 1975; Mishra and Dixit, 1977a,b,c; Rao et al., 1977; Satyanarayana and Rao, 1977; Adam and Rashad, 1984; Thakur, 1993 and Thippeswamy et al., 2003.

Previous records, viz. Deshpandey and Tipnis, 1977; Kubo and Nakinishi, 1979; Pawar and Singh, 1993; Singh, 1993 and Verma and Dubey, 1999, suggest that a measly 2% of total higher plants have been screened for pesticidal properties, and it has been found that extracts of plants belonging to
157 families are significantly toxic against micro-organisms. Of them, only 20% exhibit noticeable or prominent fungitoxic behaviour (Sehgal, 1961). Gilliver (1947) tested the extracts of 1915 flowering plants and reported that 23% of 113 families contained antifungal characteristics. Osborn (1943) studied the toxicological behavior of 2300 plant species belonging to 166 families, against bacteria. He observed that only 63 genera exhibited toxicity against *Staphylococcus aureus* and *Escherichia coli*. The insecticidal (pesticidal) properties of some plant oils were tested and established by Ahmed *et al.*, 1988; Khaire *et al.*, 1992; El-Nahal *et al.*, 1997; Shaaya *et al.*, 1997 and Verma and Dubey, 1997.

The beginning of the twentieth century ushered in a new line of research - the study of the antimicrobial efficacy of various plants and their components. In the second half of that century tremendous work has been done to study the efficacy of different plant extracts, in different aspects. A large number of research scholars have time and again studied the numerous activities of these products. Sehgal (1961) observed that a large number of plant extracts have food preserving properties. The fungitoxic properties of plant extracts were established by – Bhargava *et al.*, 1981; Pandey *et al.*, 1983; Nath and Bordoloi, 1984; Singh and Pandey, 1989; Sundriyal, 1991; Mishra *et al.*, 1993; Kelasthane and Lakpale, 1994.
Furthermore, the antifungal activity of some higher plant extracts has been established. The components of these extracts have been isolated, purified and tested by several workers viz. Kumar and Nene, 1963; Nene et al., 1968; Jain and Pathak, 1970; Nene, 1971; Shekhawat and Prasad, 1971; Gupta et al., 1972; Khanna and Chandra, 1972; Sheikh & Agnihotri, 1972; Bambode & Shukla, 1973; Dixit and Misra, 1974 a,b,c; Dixit and Singh, 1974; Gilliver, 1974; Singh, 1974; Dixit et al., 1975; Egawa et al., 1977; Marini-Bettalo, 1977; Misra and Dixit, 1977a,b,c; Narain and Satapathy, 1977; Ahmed and Agnihotri, 1978; Chaumont and Bourgeois, 1978; Kasmi and Trivedi, 1978; Singh and Sharma, 1978; Kapoor et al., 1981; Srivastava et al., 1984; Nath et al., 1986; Tripathi et al., 1986; Natarajan and Lalithakumari, 1987; Ganeshan, 1994; Kelasthane and Lakpale, 1994; Ganeshan and Krishnaraaju, 1995; Singh and Singh, 1997 and Saoji and Baitule, 1998.

Moreover, in India, Narain (1971) has emphasized the efficiency of crude plant extract as antibiotics on different plant disease causing microorganisms. Banerjee and Sen, 1979; Mahadevan, 1982 and Daswani and Bohra, 2003, have studied the antibiotic activity of plants and their extracts. Outside India, Bocher (1938) was a pioneer in studying the antibacterial efficacy of plant extracts. Similar work was also conducted by Osborne, 1943; Baer et al., 1946; Mitscher et al., 1972 and Aizeman, 1978. It can be safely concluded that plant extracts
and their components have effective antifungal, antibiotic or pesticidal properties in different concentrations. They are also effective against a horde of microorganisms causing both plant and animal diseases. An uncountable number of plant species form a part of our daily diets and each of them has some nutritive or medicinal values. Indian dishes include a number of spices and condiments, the most commonly used spices being – onion, garlic, ginger, mustard, coriander, cumin etc. These spices have characteristic pungent odors due to the presence of volatile oils in them. Purohit et al., 2002, have also studied the antifungal activity of various spice plants. Recent studies have shown that the volatile constituents (essential oils) of many plants and their component parts are effective mycotoxicants. In India, several workers have reported the antifungal nature of the essential oils of various plants viz. Garg, 1947; Gautam and Purohit, 1974; Avadhoot and Verma, 1978; Chaurasia and Kher, 1978; Misra and Dixit, 1978 a, b & 1980; Narayan et al., 1980; Kapoor et al., 1981; Dixit et al., 1983; Saxena, 1984; Jaishwal, 1990; Kishore et al., 1993; Rao and Srivastava, 1994; Singh et al., 1995; Nath et al., 1996; Pandey and Dubey, 1997; Singh et al., 1997; Dubey et al., 1998 and Pradeep et al., 2003. Outside India, the antifungal property of essential oils of plants have been studied and confirmed by – Walker et al., 1937; Wollmann et al., 1973; Crison and Hodison, 1978; French, 1985; Thompson, 1989; Grace, 2002 and others. Taxonomists often use the presence of essential
oils in plants as an identifying character in plant classification. Among the genera possessing essential oils, many of them belong to the following families – Myrtaceae, Rosaceae, Poaceae, Zingiberaceae, Asteraceae, Lamiaceae and Brassicaceae.

Studies on Zingiberaceae:

Some members of the Zingiberaceae family find constant use in the kitchen as spices, as most members possess strong pungent odors & many of them yield essential oils. Two genera of the family, which are very commonly used as spices, are – Curcuma and Zingiber, but not all species are known to be edible. The most studied genera and species are  *Curcuma longa* Linn, *Alpinia spp* & *Zingiber spp*.

The genus Zingiber, having a numbers of species, is represented by a rhizomatous herb. Most species possess an essential oil. *Z. officinale* Rosc. is cultivated for consumption. *Z. zerumbet* (L) Smith, though not consumed in India, has been reported to be consumed in different parts of the world. The ginger of commerce, *Z. officinale*, has a pleasing and stimulating effect on the olfactory lobes. This is because of the volatile nature of its essential oil. It is used regularly as a spice, which ascertains that it is not toxic to mammals.
Ginger has been used as a medicine since ancient times, recorded in early Sanskrit and Chinese texts and ancient Greek, Roman and Arabic medical literature (Bone, 1997). The Ayurvedic Pharmacopoeia specifically recommends ginger for flatulent intestinal colic (Karnick, 1994). The Chinese pharmacopoeia lists ginger for epigastric pain accompanied with cold feeling, vomiting and diarrhea (Tu, 1992). In Germany, it is officially approved as a component of antiemetic stomach medicines (Wichtl and Bisset, 1994; DAB, 1997; Meer-Buchtela, 1999). In the U.S.A., it is used as a main component of digestive, antinausea, cold and flu dietary supplements. It is also used extensively by the Ayurvedic and traditional Chinese medicinal herbal teas and by licensed acupuncturists and naturopathic physicians. The King’s American Dispensatory indicates ginger in the loss of appetite, flatulence, borborygmus (rumbling or gurgling sound of gas in the intestines), spasmodic gastric and intestinal contractions and cool extremities (Taber, 1962; Felter and Lloyd, 1983; Leung and Foster, 1996).

The rhizome of *A. zerumbet* Pers. is used as a substitute for that of *A. galanga* and even ginger. The essential oil obtained from it is active against *Candida albicans*. The plant extract is anti-ulcerative and spasmolytic. The rhizomes are used as an aromatic stimulant and carminative. A flavonoid isolated from the rhizome shows significant antifungal activity against a variety
of pathogenic fungi like *Trichophyton rubrum*, *T mentagrophytes*, *Epidermophyton floccosum*, and pathogenic and non-pathogenic yeasts. It also has antibacterial properties against Gram +ve and -ve bacteria.

Modern human studies have investigated ginger as an antiemetic- (Philips *et al.*, 1993), anti-nausea treatment (Pace, 1987), a prophylactic against motion sickness (Mowrey and Clayson, 1982; Stott *et al.*, 1985; Riebenfeld and Borzone, 1986; Helmont *et al.*, 1989; Stewart *et al.*, 1991), a treatment for vertigo (Grontved and Hentzer, 1986) and for its effects on human platelet function (Srivastava, 1989; Verma *et al.*, 1993). The above publications and research works support the safety, non-toxic nature and efficacy of ginger as a medicine.

*Zingiber* enjoys a wide distribution in the Old World Tropics, mainly in India, Eastern Asia and Malaysia. Altogether fifteen species have been reported to occur in India, some cultivated and others wild. *Z. zerumbet*, though wild, is reported to be used as a medicine for the treatment of cough, stomachache, asthma, worms and in leprosy and other skin diseases (W.O.I., vol.ix).
Among the fifteen species found all over India, eight are found to be growing abundantly all over the forests of Assam and also under cultivation in fields for commercial purposes. The species *Z. officinale* Rosc. is used regularly as a spice all over Assam. The two species, *Z. officiale* Rosc. and *Z. purpureum* Rosc. are edible and have great commercial value. *Z. zerumbet* (L) Smith grows wild in the forests and can also be cultivated. Even though reports suggest that the species is consumed in certain areas of the world, in the tropical areas of Asia, the rhizome and its extracts are used medicinally for the treatment of cough, stomachache, asthma, worms, leprosy and other skin diseases. Another species *Z. capitatum* Roxb also grows all over Assam. The other four species growing in Assam are – *Z. rubens* Roxb, *Z. intermedium* Baker, *Z. clarkei* Roxb and *Z. marginatum* Roxb.

An important character of *Zingber*, is the presence of essential oils, in varying amounts in the different species. The essential oil lends a characteristic flavor to a particular plant species. The essentials oils of *Z. officinale* Rosc. and *Z. squarrosum* Roxb, have been tested against few *Aspergillus* species, and it was observed that there is slight inhibitory effect (Singh et al., 1997).

In Assam, these plants, i.e members of Zingiberaceae grow luxuriantly due to a very conducive climate. The climate of Assam is hot and
very humid; with a high degree of moisture content all round the year. The high humidity also pampers the growth of another group of plants, i.e. the fungi, which grow and multiply rapidly due to the hot and humid climate. The fungal thalli are composed of the ramifying mycelia and the erect sporangiophores, which bear the sporangia or spore producing bodies. The spores are light and microscopic, being carried away from place to place by the wind. They impart a characteristic color to the fungal colonies. The percentage concentration of spores in the air varies from place to place and also within the same area depending on the weather (rainfall, temperature, humidity, wind speed and velocity etc.), time of the year (i.e. season), time of the day, activities in the area etc. The study of the atmospheric microflora is known as aerobiology.

**Aerobiological studies:**

Aerobiology, which deals with the study of the biological components of the air, is a recent branch of biological studies. Aerobiological studies have revealed that a number of living and non-living components are present in the air.

Numerous research works have been carried out to study the components of the air and it has been established that it is loaded with fungal
spores, pollen grains, algal cells (unicellular, microscopic) dust particles, dead cells etc.

Various aspects of aerobiology have been studied, both positive and negative. Among the latter, allergic disorders merit special attention. Anton von Pirquest coined the term ‘allergy’ in 1906 (cit. Tilak, 1989). It has been derived from two Greek words ‘Allos’ (different or change) and ‘Ergos’ (work or action). Allergy is defined as altered reactivity to a substance that is harmless in itself but induces the synthesis of antibodies in susceptible human beings on first and subsequent exposures. Antigens responsible for such reactions are known as allergens. A constitutional defect in some people result in the production of certain sensitizing antibodies by their immune systems. The antibodies are produced due to the exposure to some allergens like pollen grains, fungal spores, feathers, chemicals etc. Studies have revealed that mediators of allergy are released due to allergen-antibody reaction. The mediators bring about a precipitation of symptoms of allergic disorders such as breathlessness in asthma patients, sneezing and running nose of rhinitis patients and rashes in patients suffering from urticaria etc. The human system is known to possess proteins with antibody properties. These proteins are known as immunoglobulin, which are divided into five classes, namely- IgG, IgE, IgM, IgD and IgA, of which the first two, i.e. IgG and IgE, play an important role in
atopic allergy. Atopic allergies are localized anaphylactic reactions, which sensitize the susceptible patients spontaneously. The offending allergens include, among others, mould spores, pollen of grasses and other trees, various kinds of food such as milk, eggs etc. IgE antibodies possess the unique property of binding mast cells and basophils. On contact with an allergen, the cell bound IgE antibodies mediate the release of active amines, like histamine from the mast cells and basophils, causing symptoms of allergy. Depending on the nature of exposure, allergens are classified as– a) inhalants b) injectants c) ingestants and d) contactants. Coombs and Gell (1963) classified allergenic reactions into four types– Types I, II, III and IV. The Type I hypersensitivity reactions are mediated by IgE class of immunoglobulins. Allergenic rhinitis and asthma is a couple of examples of this type of hypersensitivity reaction. It has been reported that an estimated 15-20 % of the world population suffers from major allergic diseases, while in India; it is more than 10% of the population (Singh and Malik, 1992). A recent study by Singh & Singh (1999), has suggested that the scenario in Delhi is becoming alarming, with an estimated 60% of its workers suffering from respiratory disorders. The concentration and incidence of pollen grains and fungal spores vary from place to place. The fungal spores are responsible for a number of allergic disorders, like asthma, eczema, dermatitis etc. Most prominent among the fungal species causing allergies are
the numerous *Aspergillus species*. They cause a series of disorders, which are collectively termed as aspergilloses.

Several workers have tried to study the aeromycoflora of different areas, the world over. Their studies have revealed that, air is a storehouse of varied fungal species. Also it has been established that they are responsible for a number of disorders in plants and animals including man.

**Studies in India:**

Cunningham (1873) initiated an aerobiological survey of the atmosphere of Calcutta, for the first time in India. Through his studies, he tried to establish a correlation between the number and types of microbes and the diseases affecting the masses. His studies were not followed up by any other works for a long period of time. After a gap of almost 80 years, Kashhiwal *et al.*, 1958; carried out aeropalynological studies in Jaipur. But in the following period, that is after 1958, a lot of work has been done in different parts of India, to study the aeromycoflora. Even then, till 1978, there was no co-ordinated national programme on aerobiological studies. But in February 1978, an “All India Co-ordinated Project on Aerobiology” (AICP) was chalked out, organized and implemented under the joint auspices of the National Botanical Research Institute (NBRI) Lucknow and the Council
of Scientific and Industrial Research (CSIR) Centre for Biochemicals. P.K.K. Nair of NBRI, Lucknow was conferred the duties of coordinator of the Project. The A1CP was planned and jointly proposed by three laboratories and financed by CSIR. Aero-biologists from different parts of India met at the Bose Institute, Calcutta in 1980, at the ‘Workshop on Modern Trends in Aerobiology with particular reference to Plant Pathology and Medicine’. During the workshop, the ‘Indian Aero-biological Society’ (IAS) was formed, which started from 31st January 1980 (Chanda and Gupta, 1989). Recently, the Ministry of Environment and Forests, Govt. of India, New Delhi sponsored an AICP, on – “Aeroallergence and Human Health”, to emphasize on the aerosols of biological origin, especially pollen and fungal spores present in the outdoor and indoor environments. It came into effect from 1994. Dr. A.B. Singh, CBT, New Delhi, was the coordinator of the project. The Deptt. Of Botany, Gauhati University, was one of the centers of the above mentioned project.

In India, there was a spurt of aerobiological studies in the second half of the twentieth century. Work has been carried out by a number of scholars in this field- viz. – Bhagwan and Pande, 1957; Nair, 1963; Agarwal et al., 1969; Bhati and Gaur, 1972; Chakravorty, 1974, ’76; Shivpuri, 1980; Mandal and Chanda, 1981; Agashe and Anand, 1982; Chakraborty et al., 1982; Tilak and Saibaba, 1986; Agarwal et al., 1987; Singh et al., 1987; Tilak, 1987, ’89; Jain
and Mishra, 1988; Sarma and Sarma, 1993; Satheesh et al., 1993; Agashe and Sudha, 1995; Ghani, 1996; Rastogi and Chandel, 1996; Rafiyuddin et al., 1997; Raha and Bhattacharya, 1997; Agashe and Anuradha, 1996, '98; Agashe and Mathew, 1998; Singh et al., 1998; Pugalmaran and Vittal, 1999; Singh and Singh, 1999; Singh and Rakhi, 2003, Majumdar and Hazara, 2005 and Upadhaya and Jain, 2005.

Studies Abroad:

Outside India aerobiological work has been done by a large number of workers, a few of whom are – Gregory, 1952, '73: Ogden and Lewis, 1960; Adams and Hyde, 1965; Ordman, 1970, '72; Bassett et al., 1978; Chamberlain, 1982; Geimeinhardt and Wallenstein, 1985; Solomon, 1988; Edwards et al., 1990; Lacey, 1991; Gallo, 1993; Mizuki et al., 1994; Li and Kendrick, 1995; Lucas et al., 1999; Kontoyiannis et al., 2002 and Steinbach, 2003.

Studies in Guwahati:

Aerobiological studies have also been carried out in Guwahati by – Baruah, 1961; Baruah and Chetia, 1966; Bora and Baruah, 1980; Sarma and Sarma, 1993; Bora et al., 1996; Devi et al., 1996; Devi et al., 2002 and
Das et al., 2002. Their studies have revealed the high incidence of fungal spores and pollen grains in the atmosphere of Guwahati, all round the year.

Considering all the above factors and after review of all the available literature, a conscious effort has been made to carry out the following research works:

1) To make a survey of the available *Zingiber* species in Assam and selection of test plants.

2) To extract the essential oils of the selected *Zingiber* species, i.e. *Z. officinale* Rosc., *Z. zerumbet* (L) Smith., *Z. capitatum* Roxb. and *Z. purpureum* Rosc.

3) To make an aeromicrobiological survey to study the aeromycofloral composition of Greater Guwahati.

4) Selection of test fungal species viz. *Aspergillus niger*, *A. nidulans*, *A. flavus* and *A. fumigatus*.

5) Testing the antifungal efficacy of the Zingiber oils on the selected test fungi.