CHAPTER - 1

INTRODUCTION
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1.1 DRUG RESISTANT MICROBES

The search for selective antimicrobial agents has gained momentum in recent years due to the growing cases of microbial resistance to the time honoured antibiotics (Anderson and Irobi, 1994). The use of antibiotics is losing the effectiveness since many of the pathogenic microbes especially bacteria and fungi have developed substantial resistance to the antibiotics (Jones, 1998; Sushilkumar, 1998; Austin et al., 1999). Moreover the widespread use of antibiotics has led to the decimation of sensitive organisms from the population with the consequent increase in the number of resistant organisms. For instance, after the World War II, rapid development of antibiotics began to increase which ultimately led to the evolution of drug resistant microbes especially bacteria in particular on an alarming rate. At present there is a list of top ten drug resistant microbes which includes Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Streptococcus pneumoniae, Shigella dysenteriae, Neisseria gonorrhoea, Mycobacterium tuberculosis, Enterococcus sp., Haemophilus influenzae and Enterobacter aerogenes. These microbes are resistant to well known antibiotics such as penicillin G, tetracycline, erythromycin,
chloramphenicol, vancomycin and sulfonamides and hence known as multiple drug resistant strains (Purohit, 1977).

1.2 NEED FOR EFFECTIVE MICROBIAL AGENTS

Microbial resistance to antibiotics represents a serious problem for clinicians since most of the rampant killer diseases are of microbial origin and account for high proportion of mortality in underdeveloped as well as developed nations (Gundidza and Gaza, 1993; Jones, 1998; Guilletmot, 1999). A decade ago in India, typhoid could be cured with three inexpensive drugs namely Cephalosporins, Penicillin-G and Chloramphenicol. But today these drugs are largely ineffective against the life threatening typhoid fever. In Eastern Europe and parts of Russia, more than 10 per cent of tuberculosis patients cannot be cured completely because of the drug resistant strains which are insensitive to the most powerful antibiotics viz. Streptomycin and Rifampin. In the US alone about 14000 people die every year because of drug resistant microbes which infect people in hospitals (Nosocomial infections). In much of the South-east Asia 98 per cent of gonorrhea causing strains have become resistant to Penicillin, which had been the first line treatment for decades (Kaufman, 2000). Therefore there is an urgent need for the discovery of alternative, safer and more effective antimicrobial agents in order to control the life threatening pathogens.

1.3 TRADITIONAL MEDICAL PRACTICES

Ethnopharmocological literatures have reported several medicinal plants which have been used by traditional people all over the world for treating wounds, cuts, dysentery, diarrhoea, coughs, sore throats, fever,
jaundice, skin ailments and venereal diseases (Tanira et al., 1994). It has been inferred from the ethnopharmacological literature that more than 180 different species of medicinal plants were used by the traditional medical practitioners and many of the plants are in use even today. Reports have also shown that in many developing countries as many as 80% of the available medicines are obtained mainly from medicinal plants (Penso, 1980; Alade and Irobi, 1993). India is said to be the paradise of herbs in the world. All varieties of herbs, spices and aromatic plants are found growing in India. India has a long history on the use of large number of medicinal and aromatic plants for various purposes. Our ancient literature right from Atharva Veda provides ample references on native plants and their properties to alleviate human sufferings and for promoting a long and healthy life (Rao, 1991). Our ancient texts mention about 700 medicinal plants which were used in the preparation of medicines. It is estimated that there are more than 4000 manufacturers of Ayurvedic medicines in the country who prepare nearly 15000 preparations to deal with various health problems (Anonymous, 1991).

1.4.1 Bioactive Principles in Plants

Medicinal plants are known to possess many potentially valuable therapeutic agents that provide raw materials for the preparation of medicines (Fernandez, 1995). Although plants lack immune response they certainly have an inbuilt system for protection against biotic and abiotic stress conditions. As they coevolved with pathogens, in course of time they also developed some chemicals which protect them against the pathogens. Therefore it is reasonable to expect a variety of phytochemicals with
specific or general antimicrobial potentiality. The bioactive substances in plants are produced as secondary metabolites which may be stage specific or organ or tissue specific. In fact there are several studies which revealed the presence of some compounds with antimicrobial properties (Cowan, 1999). The roots, stems, barks and leaves of several plants have been widely evaluated for such bioactive compounds and the results obtained proved that these compounds are the sources of new drugs, antibiotics and agrochemicals. These bioactive compounds are known as phytochemicals and are exploited on a large scale because of their less toxic, more systemic and easy biodegradability. Therefore during the last few decades there has been a resurgence of interest in plants as sources of medicines and of novel molecules for the treatment of microbial infections (Kinghorn, 1987; Williamson et al., 1996).

1.4.2 Natural Products and Potency

It is well known that the antimicrobial spectra of natural products isolated from higher plants are comparatively narrow but their potency is often reasonable. They are comparatively easy to synthesize and the synthetic analogues can possess enhanced therapeutic potential. Therefore antimicrobial research on plants is geared towards the discovery and development of new antibacterial and antifungal agents for human and or agricultural use (Kinghorn, 1987). Several countries have already started this work and the local medicinal plants are being screened for possible antimicrobial properties.
1.4.3 Drug industry and herbal depletion

World Health Organisation (WHO) estimated that as many as 80% of the developing world’s population depend on traditional medicine for their primary health care needs. The greater part of this traditional therapy involves the use of plant extracts and their active principles. The drug industries therefore harvest the plants on a large scale. The Indian herbal drug industry for example collects 90% of its plants from the wild and more than 70% of these plant drugs manufacture involves destructive harvesting of the plants. As a result of this over exploitation and habital destruction, several medicinal plants are being lost day by day from our biosphere.

1.5 Micropropagation

Plants have been the traditional source for raw materials and finished medicinals since the dawn of civilization. A rich heritage of knowledge on preventive and curative medicines was even available in scholastic work included in the Atharva Veda, Charaka, Susrutha, etc. An estimate suggests that about 13000 plant species are known to have worldwide use as drugs. In India, out of 15000 species of flowering plants, about 17% are considered to be of medicinal value (Jain, 1968). Among several plant species known for their medicinal uses, phytochemical tests have been performed in about 5000, and nearly 1100 species are extensively exploited in 80% of Ayurvedic, 46% of Unani and 33% of Allopathic medicines (Gouri, 1996). It is reported that 41% prescriptions in USA and 50% in Europe contain constituents from natural products. This trend of using natural products is increasing (Shah, 1982). Moreover natural products have also served as
models for modern synthetic drugs, such as atropine for tropicamide, quinine for chloroquine and cocaine for procaine and tetracaine. In fact, active plant extract screening programmes continue to result in new drug discoveries.

In view of the growing world population, increasing anthropogenic activities, rapidly eroding natural ecosystems etc., the natural habitats for a great number of herbs and trees are dwindling. Many of them are facing extinction (Houghton, 1997). To cope with this alarming situation, the recent exciting development in biotechnology has come as a boon. The powerful techniques in plant cell and tissue culture, recombinant DNA, bioprocess technologies etc. coupled with most sophisticated analytical tools such as NMR, HPLC, GC-MS, LC-MS, etc. have offered mankind the great potency of exploiting the totipotent biosynthetic and biotransformation capabilities of plant cells under *in vitro* conditions (Stockigt *et al.*, 1985). The scope for *in vitro* germplasm preservation and large-scale production of plant secondary metabolites has brightened.

1.5.1 Problems of collecting medicinal plants from the wild

Only a few medicinal plants of high economic value like *Atropa belladona*, *Vinca rosea*, *Cinchona* sp., *Dioscorea* sp., *Papaver somniferum*, *Ruta graveolens* have been cultivated under field conditions. The majority of plants used for medicine are harvested from the wild. This results in several serious problems such as depletion of resources, extinction of rare species, material not being available in large quantities and throughout the year, incorrect identification and adulteration of the plant material.
1.5.2 Unavailability of material throughout the year

The plants growing in the wild are dependent on soil, seasons and weather conditions and, hence, they may not be available throughout the year. *Curculigo orchioides*, a herbaceous plant with anti neoplastic properties is found only during the rainy season. When the monsoon season is over not only the shoots dry up, but also the subterranean rhizomes shrivel and cannot be collected.

1.5.3 Need for cultivation of medicinal plants

1.5.3.1 Possibility of large scale, timely supply of material of assured quality

If efforts are made for systematic cultivation of medicinal plants instead of collecting them from the wild, many of the problems mentioned above will be minimized. Properly identified and certified planting material can be supplied to the growers. Cultivation of the plants can be planned to meet the needs of the industry in required quantities and at the required time. Unintentional adulteration can be avoided and it will be easier to check deliberate adulteration.

1.5.3.2 Plant tissue culture

Plant tissue culture is a technique for growing isolated cells, tissues and organs in a defined nutrient medium under aseptic condition (*in vitro*). The techniques are characterized by the following:

1. They occur on a microscale that is on a relatively small surface area.
2. The environmental conditions are optimized with regard to physical as well as nutritional and hormonal factors.
3. All microorganisms as well as other pests of higher plants are excluded.

4. The normal pattern of plant development often breaks down, and an isolated tissue can give rise to a callus or can develop in many universal ways (e.g. organ formation, somatic embryogenesis, etc.) (Pierik, 1987).

The intricacies involved in the micropropagation of medicinal plants have been completely elaborated periodically by Evans et al. (1981a, b), Ammirato (1983), and Bajaj (1986). These and several other reports establish the efficacy of the application of in vitro techniques that can profitably be utilized for the regeneration and temporal monitoring of secondary metabolites in plants.

1.5.3.3 Reasons for choosing *Momordica dioica* for micropropagation

*Momordica dioica* is a popular vegetable in India, Bangladesh, Sri Lanka and other neighbouring countries. The fruit contains a high amount of vitamin C, as much as 247 mg/100 g edible portion (Bhuiya et al., 1977) and the plant as a whole is medicinally valuable. The young twigs and leaves of this crop are also used as vegetable (Fakir et al., 1992). As a result, its popularity in domestic as well as export market has increased sharply during the last decade. Hence commercial cultivation of this plant has also been expanded (Hoque et al., 2000).

Improvement of this vegetable crop has not been attempted perhaps because of its dioecious nature and vegetative mode of propagation (Ali et al., 1991). Germination of its seed is very difficult or impossible because of
the hard seed coat (Rashid, 1976). Moreover it is not possible to predict the sex of the seed-produced plants. Conventional techniques of reproduction are often tedious and impractical on a large scale. So the only possibility of propagation of this plant is through micropropagation. The application of tissue culture method for large scale propagation of cucurbitaceous vegetable taxa has been well demonstrated (Moreno and Roig, 1990; Dong and Jia, 1991; Debeaujon and Branchard, 1992; Misra and Bhatnagar, 1995; Islam et al., 1999).