DISCUSSION
Some of the oldest traditional medical systems include Chinese, Ayurvedic, Unani, Japanese and recently added homeopathy and chiropathy that is also around 200 years old. The use of traditional medicine includes:
(i) medication by use of medicinal plant, minerals, animal materials and
(ii) non medication: acupuncture and yoga.

India with its varied climate, soils and agro-ecology possesses immense plant diversity, with over 15,000 species of higher plants. Both our Indian civilization as well as our diverse tribal heritage has gone a long way in conserving the wild weedy species, native land races and primitive cultivars.

The potential of plants as sources of medicine is often taken in support of identification and preservation of the world’s most species rich ecosystems. Such assessments are speculative and totally based on chance. Screening the vast vegetable world for potential sources of medicine and their use in the traditional way or through the application of biotechnology for a large scale industrial production of the active constituents or by chemical synthesis is a very uncertain and a long term proposition that involves heavy financial investment with no certainty of the economic returns. There is no guarantee that the future drugs will all be derived from plants.

Traditional medicine has been described by the World Health Organisation (WHO) as one of the surest means to achieve total health care coverage of the world's population. In spite of the marginalisation of traditional medicine practised in the past, the attention currently given by governments to widespread health care application has given a new drive to
research, investments and design of programmes in this field in several developing countries.

Most developing countries are endowed with vast resources of medicinal and aromatic plants. These plants have been used over the millennia for human welfare in between man and his environment continues even today as a large proportion of people in developing countries still live in rural areas. Furthermore, these people are precluded from the luxury of access to modern therapy, mainly for economic reasons.

The demands of the majority of the people in developing countries for medicinal plants have been met by indiscriminate harvesting of spontaneous flora including those in forests. As a result many plant species have become extinct and some are endangered.

Numerous medicines have been derived from the knowledge of tropical forest people and clearly there will be more in the future. This alone is a reason enough for any and all programmes to be concerned with the conservation, development, and protection of tropical forest regions. Human needs and problems are primary component of any conservation programme. It is therefore necessary that systematic cultivation of medicinal plants be introduced in order to conserve biodiversity and protect threatened species. Systematic cultivation of these plants could only be initiated if there is a continuous demand for the raw materials.

This focus on human needs requires assessing the importance of regional forests in traditional systems of medicine, and it also requires provisions that allow for any activities to have minimal negative impact on the accessibility to these medical resources. The documentation of medicinal
uses of plants is becoming increasingly urgent because of the rapid loss of
the natural habitat for some of these plants due to anthropogenic activities.

Many plants are used for their therapeutic values and this has a
twofold effect on the world’s flora. On one hand, the demand for herbs,
particularly in parts of India, has brought some plants near extinction. Even
the simplest plant may have a future importance that we cannot predict.
Efforts to develop drugs from medicinal plants should address diseases and
health problems seen in developing countries as well as diseases which
primarily affect developed countries’ population. Saving the world's plant
resources calls for more protection and management, more research, and an
increasing level of public awareness about our vanishing heritage.

Indigenous and local communities are concerned that the rate of
knowledge erosion has never been so high, as it is in the current generation,
and that such knowledge erosion poses an even more serious threat to the
conservation of biological diversity than resource erosion. There is,
therefore, an urgent need to formulate an array of incentive measures to
ensure that members of the younger generations will want to learn, value,
adapt and apply the traditional knowledge, innovations and practices of their
elders.

Within the framework of the management and conservation of
biological diversity, it is worthwhile noting that at this level, no exhaustive
plan of control and evaluation of the resources of medicinal plants has yet
been proposed.

The results for the general screening for antibacterial and antifungal
activity were exhibited by the extracts of the plants studied. In the initial
screening the leaf extracts of the commonly found leguminous plants were
used, it was clear that most of the plants possess anti-microbial activity with few exceptions. However, there was slight variation in the activity of the plant extracts. The dried powders of the leaves were extracted in distilled water and were used in the initial screening.

It was clear that *Acacia nilotica* and *Abrus precatorius* leaf extracts exhibited maximum activity against the selected test bacteria as well as fungi. So these two plants were selected for further studies. The test system to assess the antimicrobial properties of the extract was extended to the bacterial and fungal pathogen of plants as well as human beings.

The effect of extracts of *Acacia nilotica* and *Abrus precatorius* for antibacterial and antifungal activities against selected human bacterial and fungal pathogens *in vitro* was undertaken. The study was limited to extracts of *Acacia nilotica* and *Abrus precatorius* from four different plant parts viz. leaves, bark, roots and seeds. The antimicrobial activities of the *Acacia nilotica* and *Abrus precatorius* leaves, bark, root and seeds varied distinctly. Generally the extracts, irrespective of its plant parts, showed appreciable antimicrobial activities against all the tested microorganisms. It is clear that the leaf extract of both the plants showed activity against all the test microorganisms. However, the activity of leaf was relatively less than the activity of bark of the plant. Similarly, the activity of bark was comparatively less than the root extracts while the seed extracts possess the highest activity in both the plants. These results confirm the observations made by various workers with different plant and plant parts and test systems (Cotton, 1996; Taylor, 1996; Grierson and Afolayan, 1999).
In this study, the powders of various plant parts were initially extracted in distilled water, the aqueous extract showed considerable activity against all the test microorganisms. But these powders were subjected to extraction using two mostly used solvent in phytochemistry viz. Ethanol and ethyl acetate. The effect of extraction using two different solvent lead to enhance activity of these extracts. The activity in ethanol extract was higher than aqueous extraction, while ethyl acetate extraction gave greater activity than that of alcoholic extracts. This was true for all the plant parts used for both the plants.

Initial screenings of plants for possible antimicrobial activities typically begin by using crude aqueous extractions and then followed by alcohol and then by various organic extraction methods. Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol extraction. In fact, many studies avoid the use of aqueous fractionation altogether. The exceptional water-soluble compounds, such as polysaccharides (e.g., starch) and polypeptides, including fabatin (Zhang and Lewis, 1997) and various lectins, are commonly more effective as inhibitors of pathogen adsorption and would not be identified in the screening techniques commonly used. Occasionally tannins and terpenoids will be found in the aqueous phase, but they are more often obtained by the treatment with less polar solvents.

Recently, Eloff (1998) examined a variety of extractants for their ability to solubilize antimicrobials from plants, as well as other factors such as their relative ranking as biohazards and the ease of removal of solvent from the fraction. The study records similar observations made by different
workers (Estevez-Braun et al., 1994; Taylor et al., 1996; Brandao et al., 1997; Chariandy et al, 1999; Bhavnani and Ballow, 2000).

Greater and remarkable antibacterial activities of the extracts of both the plants tested were recorded with *Staphylococcus aureus* and *Corynebacterium* sp. whereas *E. coli* and *Xanthomonas malvacearum* were comparatively less inhibited. Antibacterial activities of the extracts of the *Abrus precatorius* followed by *Acacia nilotica* were most observed with *S. aureus*.

These extracts showed better activity against the Gram-positive than the Gram-negative bacteria. The results support the observation that Gram-negative bacteria are more resistant, probably because of their thick murein layer which prevents the entry of inhibitors (Martin, 1995). Similar observations were also made in the screening of two hyacinthaceae member against Gram-positive and Gram-negative bacteria (Sparg et al., 2002).

The water extracts of *Abrus precatorius* and *Acacia nilotica* inhibited both Gram-positive bacteria, and the Gram-negative, thereby suggesting a broad spectrum antibacterial property of these plant species. This seems to justify their usage for the treatment of infections by the indigenous people of this region. Water extracts show less antibacterial activity though there was more activity by these plants when extracted in ethanol and ethyl acetate. The zone of inhibition of all the test bacteria was comparable with the extracts of both the plants. The wells were filled with solution containing the antibiotics - streptomycin (500μg/ml) and tetracycline (500μg/ml) were used as the positive controls.
The concentration of the extracts 100 mg/ml being the maximum concentration tested in the experiment was much higher than the pure antibiotic with the concentration of 500μg/ml. This indicates that the extract used in the experiments contains antibacterial component more than the pure antibiotic.

Though the extract produced varied inhibition zones, the highest inhibition zone of 30 mm was produced by the extracts of AP followed by AN (28 mm). The antibacterial activity of these plants was approximately equivalent to 1 mg/ml of streptomycin and tetracycline.

The broad spectrum-antibacterial activity of some of the plants used for the treatment of various diseases appears to have justified this belief. The antibacterial activity of the plant extracts was found mainly against the Gram-positive bacteria while Gram negative bacteria appear to be resistant to the plant extracts. Similar observations have been reported by several workers (Martin, 1995; Paz et al., 1995; Vlientinck et al., 1995; Kudi et al., 1999) and are supported by this study. These observations are likely to be the result of the differences in cell wall structure between Gram-positive and Gram-negative bacteria, with the Gram-negative outer membrane acting as a barrier to many environmental substances, including antibiotics (Tortora et al., 2001).

Two of the organisms inhibited by some of the extracts, Corynebacterium and S. aureus are important food-borne pathogens, raising the possibility of using any active compounds to prevent food-borne diseases. However, the application of any compounds to medicine or food science will require safety and toxicity issues to be addressed.
Significant and better antifungal activities of the extracts of both the plants tested were recorded with *C. albicans* and *T. rubrum* whereas *A. solani* and *H. turcicum* were also inhibited. Antifungal activities of the extracts of the *Abrus precatorius* were higher followed by *Acacia nilotica*, where the activity was most observed with *T. rubrum*.

These extracts showed better activity against the human pathogenic fungi than the plants pathogenic fungi. This may be due to the reason that plant pathogen are often exposed to the plant originated antifungal compounds like phenols, flavonoids, coumarins, alkaloids etc. whereas human pathogen are new to such compounds. The results support the observation that human pathogen fungi are susceptible to plant extracts whereas plant pathogenic fungi are relatively more resistant (Chaurasia and Vyas, 1977; Himejima *et al.*, 1992; Kubo *et al.*, 1993; Suresh, 1997; Alves, *et al.*, 2001, 2003).

The broad spectrum-antifungal activity of some of the plants used for the treatment of various skin diseases appears to have justified this belief. The antifungal activity of the plant extracts was found mainly against the *C. albicans* and *T. rubrum* while *A. solani* and *H. turcicum* appear to be resistant to the plant extracts. Similar observations have been reported by several workers (Brownlee *et al.*, 1990; Apisariyakul *et al.*, 1995; Baig, 1997; Deeni and Sadiq, 2002) and are supported by this study. These observations justify their usage for the treatment of skin infections by the indigenous people.

Among fungal pathogens *Candida albicans* and *Trichophyton rubrum* were also chosen for this study since it causes serious systemic infections, including opportunistic infection in patients infected with HIV virus.
Intensive use of antibiotics often resulted in the development of resistant strains (Sydney et al., 1980).

The water extracts of *Abras precatorius* and *Acacia nilotica* inhibited both human and plant pathogenic fungi, thereby suggesting a broad spectrum antibacterial property of these plant species. This also justify their use for the treatment of skin infections by the local people of this region. Water extracts show less antifungal activity though there was more activity by these plants when extracted in ethanol and ethyl acetate. The zone of inhibition of all the test fungi was comparable with the extracts of both the plants and pure antibiotic Amphotericin B commonly used against human pathogenic fungi. The wells were filled with solution containing the antibiotic - Amphotericin B (500μg/ml) used as the positive controls. While the spore germination of plant pathogenic fungi were compared with Carbendazim (1 mg/ml) a common fungicide used in disease management of plant diseases.

The concentration of the extracts 100 mg/ml being the maximum concentration tested in the experiment was much higher than the pure antibiotic with the concentration of 500μg/ml. This indicates that the extract used in the experiments contains antifungal component more than the pure Amphotericin B with the human pathogenic fungi. Whereas for plant pathogenic fungi relatively more spore germination was observed than the pure fungicide Carbendazim. This indicates that the the extract used in the experiments contains antifungal component less than the pure fungicide.

Because of this drug resistance, the search for new antibiotics continues unabated. In this connection plants continue to be a rich source of therapeutic drugs. The active principles of many drugs are found in plants or are produced as secondary metabolites. The remarkable contribution of
plants to the drug industries was possible, because of the large number of the phytochemical and biological studies all over the world.

It indicates that several plants have the potential to generate novel metabolites. The crude extracts demonstrating anti-candidal activity could result in the discovery of novel anticandidal agents. The plants demonstrating broad spectra of activity, may help to discover new chemical classes of antibiotics that could serve as selective agents for the maintenance of animal or human health and provide biochemical tools for the study of infectious diseases.

Dhar et al. (1968) reported that the leguminaceae member were active against bacteria, fungi, protozoa and virus, similar results were observed here. These plants showing significant antibacterial and antifungal activity may be due the presence of alkaloids, flavonoids, tannin, polyphenolic and oil (Gary and Kasera, 1983; Irobi et al., 1994; Hook and Thomas, 1995; Brantner et al., 1996).

Medicinal plants in India are widely used by all sections of people either directly as folk remedies or in different indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines. It is estimated over 2000 different plants are used for medicinal preparations for both internal and external use in India alone (Anonymous, 1948-1992).

Rigveda mentions 67 plants having therapeutic effects, Yajurveda lists 81 plants and Atharveda 290 plants. The world health organization recently compiled an inventory of more than 20,000 species of medicinal plants. Indian medicinal plants and their products are used to control diverse diseases such as catarrh, bronchitis, pneumonia, ulcers and diarrhoea.
Researchers are increasingly turning their attention to folk medicine looking for new leads to develop better drugs against cancer, as well as viral and microbial infections (Galal et al., 1991; Hoffmann et al., 1993). Although hundreds of plant species have been tested for antimicrobial properties, the vast majority have not yet been adequately evaluated (Balandrin et al., 1985). In every developing country it is necessary that the documentation of medicinal plants be treated as a matter of extreme urgency (Kirtikar and Basu, 1975; Jain and DeFilipps, 1991).

The age-old tribal knowledge of plants is an important aspect of ethnobotanical research. The tribal tracts are the storehouse of information and knowledge on the multiple uses of plants. The districts of Marathwada are inhabited by a large number of different tribes. Ethnobotanically, this region has remained under explored. Vast stretches of the Marathwada region were once covered with thick natural forests. However, there has been a rapid industrialization in recent years (Naik, 1998).

In many countries such as India and China, where thousands of tribal communities still use folklore medicinal plants today to cure sicknesses. The great interest in the use and importance of Indian medicinal plants by the World Health Organisation in many developing countries has led to intensified efforts on the documentation of ethnomedical data of medicinal plants (Dhar et al., 1968; Waller, 1993; Perumal and Patricraja, 1996).

It is a necessity from the scientific point of view, to establish a rational relationship between chemical, biological and therapeutical activities of folklore medicine (Levin, 1976; Coley and Aide, 1991; Gentry, 1993). Biologically active compounds from natural sources have always been of great interest to scientists working on infectious diseases. In recent
years there has been a growing interest to evaluate plants possessing antibacterial activity for various diseases (Clark and Hufford, 1993).

A number of studies have been reported, dealing with antimicrobial screening of extracts of medicinal plants (Malcom and Sofowora, 1969; Bhakuni et al., 1974; Taniguchi et al., 1978; Moskalenko, 1986; Brantner and Grein, 1994; Grosnenor et al., 1995; Perumal Samy and Ignacimuthu, 1997). Plant derived drugs serve as a prototype to develop more effective and less toxic medicines. Hence, an attempt has been made to study the in vitro antibacterial and antifungal activity of folklore medicinal plants used in Marathwada.

A total of 42 persons from seven districts within Marathwada were interviewed, out of which 14 were well known traditional healers. The study revealed these two plant species belonging to leguminosae family are commonly found and frequently used for the treatment of various diseases. These plants were repeatedly and independently mentioned by 35 interviewees including each of the 14 traditional healers.

Based on this the plants, *Acacia nilotica*, and *Abrus precatorius* were collected for antibacterial and antifungal screening. The method of preparation which varies from one herbalist to the other, could be generally classified into three categories namely; infusions made from fresh or dried material, direct squeezing of plant leaves to extract the juices and grinding into powdery forms. However, plant parts were used in more than one method of preparation. The most frequently used parts of the plants for the treatment of various diseases were reported to be roots, leaves and seeds, constituting about 53% of the preparations. Fruits and leaves constitute 17% each while stem bark constitutes 12%.
In India, herbal medicines have been the basis of treatment and cure for various diseases: physiological conditions in traditional methods practiced such as ayurveda, unani and siddha. Although reports of antibacterial and antifungal activity of indigenous plants have been published from many regions (Nadkarni, 1908; Dhar et al., 1968) they have not been systematically conducted, except in a few cases, thereby leading to confusion in drawing a meaningful conclusions (Padmaja et al., 1993; Ndamba et al., 1994; Vijaya et al., 1995).

Indian folk medicine comprises numerous herbal prescriptions for therapeutic purposes which may be as varied as healing wounds, treating inflammation due to infections, skin lesions, leprosy, diarrhoea, scabies, venereal diseases, snake bite and ulcers etc. What is interesting however is that, quite often, a particular plant may be used for different diseases. For example, the decoction of *Sebastiania chamaelea* is considered to be tonic for diarrhoea in India and China (Caius, 1937) while the astringent effect of the juice is applied externally to cure leprosy. Similarly, the bark of *Terminalia arjuna* has been widely used in Indian system of medicine for a variety of purposes (Jain et al., 1992) and the bark is particularly effective useful in cardiovascular therapy (Vaidya, 1994).

The root of *Tragia involucrata* may be recommended as a diaphoretic as well as for the treatment of cold, leg and arm pain, skin eruptions, venereal diseases, blood purification and fever (Chopra, 1958). The tribals of Western Ghats (Malaiali, Irulas and Paliyan tribes) have been using plant parts of various species as therapeutical agents (Veale et al., 1992; Hook and Thomas, 1995). Against this background information and appreciating the knowledge on medicinal plants acquired by the tribals of Western Ghats, an
effort has been made in this study to evaluate the antibacterial activity of 34 plant species collected from this region based on their medicinal properties as suggested by the tribals.

The major challenge today is the discovery of plants with promising activities and the isolation of active principles. There are, however, many obstacles which seriously hamper this type of investigation. First, it is possible that a broad range of structurally diverse compounds contribute to the overall pharmacological activity of a plant extract and synergistic effects between those active principles may exist (Wagner et al., 1985). Secondly, there is an urgent need for more appropriate pharmacological models. Existing assays are quite often not reliably predictive for clinical efficacy. For a number of common diseases with unknown or multifactorial origin, no suitable pharmacological models have as yet been developed.

The active principles of the plants are not known, as no suitable pharmacological model exists. Medicinal plant research requires a multidisciplinary approach. The quality of research can only be as good as the co-operation between botanists, phytochemists and pharmacologists. A good collaboration between botanists and phytochemists has developed over the years, as numerous joint publications testify. The collaboration with pharmacologists is in general of more recent date and definitely needs to be intensified. As outlined above. There is a requirement for more appropriate assays to be used in plant screening. All plant extracts and pure compounds isolated in phytochemical laboratories should be submitted to as a wide range of bioassays as possible. Numerous plant extracts and isolates standing in phytochemical laboratories have to be tested and there are certainly many interesting activities yet to be discovered (Wailer, 1985).
To obtain some information on the active components, plant extracts were analyzed by TLC on silica gel. TLC plates were run in duplicate and one set was used as the reference chromatogram, and the other set was assayed. The compounds were assayed by more sensitive methods. The four various fraction from the both the plats were assayed individually.

The phenol fractions of the both plants were active against bacteria as well as fungi.

There is a large variety of active principles against microorganisms including essential oils, flavonoids, (Slowing et al., 1994; Lis-Balchin et al., 2000; Hernandez et al., 2000) and tannins (Scalbert, 1991; Djipa et al., 2000). The presence of flavonoids and tannins in can be responsible by antimicrobial activity (Sahar et al., 1997). Since the antimicrobial activity in species has been found with the amides, essential oil, lignans, phenylpropanoids, alkaloids, neolignans and chromene (Masuda et al., 1991; Alves et al., 2000; Costantin et al., 2001; Dorman and Deans 2000), its is possible that these compounds could be responsible by the antimicrobial properties.

All test samples have more potent inhibitory effects on gram-positive bacteria in comparison to gram-negative bacteria. According to the TLC separation, plants extracts yielded components with Rf values similar to the antibacterial compounds visible. In addition, many of these inhibition zones were associated with dark blue spots which had been detected under UV radiation. This may mean that the same compounds are responsible for the antibacterial activity in these plants. Both the plant extracts displayed some degree of antibacterial activity, in particular against the gram-positive bacteria \textit{S. aureus} and \textit{Corynebacterium sp.}
This is to be expected because the outer membrane of gram-negative bacteria is known to present barrier to penetration of numerous antibiotic molecules, and the periplasmic space contains enzymes, which are able of breaking down foreign molecules introduced from outside (Duffy and Power 2001). It was interesting that the extracts of both the plants showed response on both gram positive and gram-negative bacteria.

Simple phenols and phenolic acids. Some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring. Cinnamic and caffeic acids are common representatives of a wide group of phenylpropane-derived compounds which are in the highest oxidation state. The common herbs tarragon and thyme both contain caffeic acid, which is effective against viruses (Wild, 1994.), bacteria (Brantner, et al., 1996; Thomson, 1978), and fungi (Duke, 1985).

Catechol and pyrogallol both are hydroxylated phenols, shown to be toxic to microorganisms. Catechol has two 2OH groups, and pyrogallol has three. The site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity (Geissman, 1963). In addition, some authors have found that more highly oxidized phenols are more inhibitory (Scalbert, 1991; Urs and Dunleavy, 1975). The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more non-specific interactions with the proteins (Mason and Wasserman, 1987).

Phenolic compounds possessing a C3 side chain at a lower level of oxidation and containing no oxygen are classified as essential oils and often
cited as antimicrobial as well. Eugenol is a well-characterized representative found in clove oil. Eugenol is considered bacteriostatic against both fungi (Duke, 1985) and bacteria (Thomson, 1978).

The presence of phenols, saponins, tannins and alkaloids in plants may explain some of the antimicrobial actions extracts of these plants, since the antimicrobial actions of most of these phytochemical substances have been documented (Lewis and Elvin-Lewis, 1977; Plasuntherum and Iyer, 1982; Palacious et al., 1983; Sofowora, 1982; Ahmad et al., 1986; Deeni and Hussain, 1991). However, the antimicrobial activities demonstrated by extracts, which did not contain any of the tested phytochemical substances, could perhaps be due to the presence of other antimicrobial substance(s) not covered by the screening. For example, Cook et al. (1998) have observed, a very high level of antioxidants with the potential for anticancer properties, and invariably perhaps also antimicrobial actions.

The antimicrobial activity and the presence or distribution of phytochemical substances in these plants may be partly dependent on the host plant species. Taking into account the wide spectrum of antimicrobial activity of extracts of the plants against multiple bacteria and fungi, in addition to the occurrence of phytochemical substances of immense economic value (Lewis and Elvin-Lewis, 1977; Al-Shamman and Mitscher, 1979; Sofowora, 1982; Trease and Evans, 1987).

Heterocyclic nitrogen compounds are called alkaloids. The first medically useful example of an alkaloid was morphine, isolated in 1805 from the opium poppy *Papaver somniferum* (Fessenden and Fessenden, 1982); the name morphine comes from the Greek Morpheus, god of dreams. Codeine and heroin are both derivatives of morphine. Diterpenoid alkaloids,
commonly isolated from the plants of the Ranunculaceae, or buttercup (Jones and Luchsinger, 1986) family (Atta-ur-Rahman and Choudhary, 1995), are commonly found to have antimicrobial properties (Omulokoli, et al., 1997). Solamargine, a glycoalkaloid from the berries of Solanum khasianum, and other alkaloids may be useful against HIV infection (McMahon et al., 1995; Sethi, 1979) as well as intestinal infections associated with AIDS (McDevitt, 1996). While alkaloids have been found to have microbiocidal effects (including against Giardia and Entamoeba species (Ghoshal et al., 1996), the major antidiarrheal effect is probably due to their effects on transit time in the small intestine. Berberine is an important representative of the alkaloid group. It is potentially effective against trypanosomes (Freiburghaus et al., 1996) and plasmodia (Omulokoli, et al., 1997). The mechanism of action of highly aromatic planar quaternary alkaloids such as berberine and harmane (Hopp, 1976) is attributed to their ability to intercalate with DNA (. Phillipson and O’Neill, 1987).

One of the undisputed functions of flavonoids and related polyphenols is their role in protecting plants against microbial invasion. This not only involves their presence in plants as constitutive agents but also their accumulation as phytoalexins in response to microbial attack (Grayer and Harborne, 1994; Harborne, 1999). Because of their widespread ability to inhibit spore germination of plant pathogens, they have been proposed also for use against fungal pathogens of Man. There is an ever increasing interest in plant flavonoids for treating human diseases and especially for controlling the immunodeficiency virus which is the causative agent of AIDS. Here, it is intended to review some recent work on plant and fungal interactions and then survey the literature on the characterisation of various plant flavonoids
as antifungal, antibacterial or antiviral agents. The majority of flavonoids recognised as constitutive antifungal agents in plants are isoflavonoids, flavans or flavanones. The recognition that a flavone glycoside, namely luteolin 7-(200-sulphatoglucoside), is an antifungal constituent of the marine angiosperm *Thalassia testudinum* is noteworthy (Jensen et al., 1998). This plant suffers periodic infections caused by zoosporic fungi such as *Schizochytrium aggregatum*. However, whole leaf concentrations of the flavone glycoside reach 4 mg ml/1 wet tissue, which is more than sufficient to reduce growth of the above fungus by 50%. The fact that the flavone is present in a water soluble form as a sulphate suggests that it may also be excreted from the plant to ward off fouling microorganisms in the marine environment.

The presence of a phenolic group in a natural flavonoid would be expected to provide antimicrobial activity and the addition of further phenolic groups might be expected to enhance this activity. Testing the effect of various flavonoids on mycelial growth in the fungus *Verticillium alba-atrum*, a pathogen of several serious wilt diseases, showed exactly the opposite was true. The most inhibitory compounds were the parent structures, flavone and flavanone, which were active at 1 and 5 ppm, respectively. Normal hydroxyl flavonoids only inhibited growth in concentrations above 5 ppm and some were ineffective even at 200 ppm. In fact, increasing the number of hydroxyl, methoxyl or glycosyl substituents resulted in the steady loss of antifungal activity (Picman et al., 1995).

Several recent papers report the regular presence of antibacterial activity among flavonoids. Thus, the retrochalcone licochalcone C (4, 40-dihydroxy-20-meth-oxy-30-prenyl) is active against *Staphylococcus aureus*
with a minimum growth inhibitory concentration (MIC) of 6.25 mg ml/1 (Haraguchi et al., 1998). Also, the compound 5, 7-dihydroxy-3, 8-dimethoxyflavone has an MIC of 50 mg ml/1 towards *Staphylococcus epidermis* (Iniesta-Sanmartin et al., 1990). Again, the substance 5, 7, 20, 60-tetrahydroxy -6-prenyl -8-lavandulyl -40-methoxy -flavanone completely inhibits the growth of *S. aureus* at concentrations between 1.56 and 6.25 mg ml/1 (Inuma et al., 1994). The above flavanone is particularly active against antibiotic-resistant strains of *S. aureus* and could have value in treating patients, who inadvertently pick up this infection while in hospital.

Worldwide Scientists from divergent fields are investigating plants with an eye to their antimicrobial usefulness. A sense of urgency accompanies the search as the pace of species extinction continues. Laboratories of the world have found literally thousands of phytochemicals which have inhibitory effects on all types of microorganisms in vitro. More of these compounds are subjected to animal and human related studies to determine their effectiveness in whole-organism systems, including in particular toxicity studies as well as an examination of their effects on beneficial normal microbiota. It would be advantageous to standardize methods of extraction and in vitro testing so that the search could be more systematic and interpretation of results would be facilitated.