Pollution is the introduction of contaminants into the environment that causes instability, harm or disorder to the ecosystem that mostly comprises of the living organisms. Before we talk about pollution, let us discuss about the term “pollutants”. Pollutants are nothing but simply the waste or contaminants which are released into the environment. In fact, it is a silent killer which is responsible for causing various diseases and many of them are also mortal. Very few of us are aware about its exact impact on our organs. Pollution does have a severe impact on human health and environment. The incidences of various diseases are becoming prominent with the increasing rate of pollution. The diseases primarily comprise respiratory disorders, cardiovascular disorders, skin infections, throat inflammation etc.

Over three-quarters of the world population relies mostly on plants and plant extracts for health care. More than 30% of the entire plant species on the earth, at one time or many times, were used for medicinal purposes. Since ages, medicinal plants have been used in daily life to treat diseases all over the world. The widespread use of herbal remedies and health care preparations such as those described in ancient texts like the Rigveda, Yajurveda, Atharvaveda, Charak, Samhita, Sushrut Samhita, have been traced to the occurrence of natural products with medicinal properties. India is floristically very rich and recognized as one of the 12 mega biodiversity countries of the world. At a modest estimate, there are at least 47,000 species of plants not including aquatic life forms, a significant proportion of which is employed for medicinal purposes in a wide variety of ways. Healthy living is the prime motto of all the human beings in ancient times and even today.

For centuries herbs were the primary means of ensuring family’s health and in many parts of the world this is still the case (Mnimh, 1997). Modern Western medicine is very much a newcomer in the history of medicine and is practiced only by a small percentage of the world’s population. It has only been in existence for about 70 years, while herbal medicine has been around for thousands of years (Van Wijk, 1990).

The discovery and application of penicillin and other antibiotics against infectious diseases led to the development of pharmaceutical industry in the second half of the last century that has done much to combat disease in man. Apart from reduced mortality and increased lifespan, it has also led to reduced affordability and availability in developing countries. The use of traditional medicine has decreased or disappeared completely in many countries and focus has been on isolating bioactive compounds for possible use in modern
medicine instead of the rational use of traditional medicines. Most products sold by drug companies are still derived from plants (Eloff, 2000). Plants have always played an important role for mankind especially as food and medicine. In the last few decades, there has been an exponential growth in the field of herbal medicine due to failure of modern medicine in providing effective treatment for chronic diseases and emergence of multidrug resistant bacteria.

**Significant findings and recommendations**

**Phytochemical screening**

The results of the phytochemical screening are presented in chapter 4. This reveals moderate concentration of reducing sugar, saponins, tannins, flavonoids and terpenoids some of which have been associated with antimicrobial activities and thus have curative properties against pathogens (Nweze, et al., 2007). The present study indicates that oral administration of successive extracts of 8 Indian medicinal plants (G. sylvestre (gudmar), A. lunulatum (Hasraj), B. laciniosa (shivlingi), T. grandis (sagwan), V. odorata (banpasha), Dashmool, S. xanthocarpum (pasarkateli), W. coagulans (paneerphal) improves the potent anti-microbial activity. The extract lowers the skin infections caused by environmental pollutions. Further pharmacological and biochemical investigations are essential to elucidate the mechanism of action.

These compounds are known to be biologically active and therefore aid the antimicrobial activities. These secondary metabolites exert antimicrobial activity through different mechanisms. Tannins have been found to form irreversible complexes with prorinerich protein (Shimada, 2006) resulting in the inhibition of cell protein synthesis. Parekh and Chanda, (2007) reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery (Dharmananda, 2003). These observations therefore support the use of these medicinal plants in herbal cure remedies. Li and Wang, (2003) reviewed the biological activities of tannins and observed that tannins have anticancer activity and can be used in cancer prevention, thus suggesting that these medicinal plants have potential as a source of important bioactive molecules for the treatment and prevention of cancer. The presence of tannins in these medicinal plants supports the traditional medicinal use of these plants in the treatment of different ailments.
Saponin was found to be present in these plants extracts and has supported its usefulness in managing inflammation. Flavonoids, another constituent of extracts, exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties (Hodek, et al., 2002). It was concluded that these plants could be a potential source of active antimicrobial agents.

**Thin Layer Chromatography (TLC) and High Pressure liquid chromatography (HPLC)**

Thin layer chromatography (TLC), also called planar chromatography, is a widely accepted and extensively used separation technique that is over 65 years old. The technique is simple, cost effective, versatile, and useable in all laboratories around the globe. It can be easily adapted to any given situation of qualitative, quantitative or preparative separation. Despite the great variety and complete automation of the technique, it still lags behind other chromatographic techniques when it comes to its use as analytical technique. However, there is no substitute for this technique for situations requiring qualitative analyses of plant extracts. TLC has nearly become indispensable for the standardization of plant materials, be it the fingerprint profiling or analysis of a marker. The advantages of the technique over other analytical techniques are many while handling plant materials. The samples can be applied without undertaking tedious, time-consuming processes of sample preparation. The loss in sensitivity is further compensated by the gain on several fronts, including ease of assays, multiple sample analyses and low cost per sample.

TLC has been regarded as a simple, rapid and inexpensive method for the separation, identification and semi-quantification of a wide variety of substances by scanning chromo-strips with or without detecting reagents, under normal or UV light. These $R_f$ values are simple, reproducible and thus reliable marker to verify the purity of the crude drugs. In view of this, TLC investigations of different plants were carried out. TLC fingerprints were generated from petroleum ether extracts of *G. sylvestre* (gudmar), *A. lunulatum* (Hasraj), *B.laciniosa* (shivlingi), *T.grandis* (sagwan), *V. odorata* (banpasha), Dashmool, using solvent system Hexane: Acetone (3:1). Petroleum ether extracts of various plants when run in solvent system Acetone: Hexane (1:3) with standard showed the presence of various standard compounds at different $R_f$ value. $R_f$ value was calculated using visual TLC, by putting in iodine chamber and by spraying with 10% H$_2$SO$_4$, spots were observed by spraying with 10% H$_2$SO$_4$. Plants showed the presence of of β- Sitosterol, Lupeol and Stigma sterol at different
R_f values and were compared with the market sample. By these fingerprints, the quality control of an authentic drug in various quarantines was achieved.

Like *G. sylvestre* (gudmar) showed the presence of Stigmasterol at R_f -.63 (Purple) and lupeol at R_f -.72 (Pink), *A. lunulatum* showed the presence of β-Sitosterol at R_f -.60 (Purple) and Lupeol at R_f -.63 (Pink), *B. laciniosa* (stem) showed the presence of Stigmasterol R_f -.63 (Purple) and lupeol at R_f -.72 (Brown), *T. grandis* (leaf) showed the presence of β- Sitosterol R_f -.56 (Purple), Stigmasterol R_f -.58 (Purple) and Lupeol at R_f -.64 (Pink), *T. grandis* (sagwan (stem) showed the presence of β- Sitosterol R_f -.55 (Pink), Stigmasterol R_f -.62 (Purple) and Lupeol at R_f -.66 (Purple), *V. odorata* (banpasha) showed the presence of β-Sitosterol R_f -.55 (Pink), Stigmasterol R_f -.58 (Purple) and Lupeol at R_f -.60 (Purple), where as Dashmool showed the presence of Lupeol at R_f -.72 (Purple), Stigmasterol at R_f -.62 (Purple) and β-Sitosterol at R_f -.60 (Pink).

HPLC is a quick, reliable and data-oriented method used for quality control of various drugs and provides sufficient characteristics that allow these to be distinguished. Previously, various TLC procedures were worked out for various drugs using petroleum ether solvent. These systems had the limitations of resolution, sensitivity and adoption for quantification; on the contrary HPLC has been the technique of choice for the separation and quantification of natural products as isocratic separations are favoured, wherever possible, since they do not require complex gradient systems, and thus, can easily be reproduced and eliminate the necessity of re-equilibrating the column between the runs. In the present study, HPLC was performed for Lupeol, β-Sitosterol and Stigma sterol run in methanol under 254 nm, the retention time time recorded at 18.138, 6.714 and 17.656.

HPLC profile of petroleum ether extract of *V. odorata* have characteristics peaks at retention time 2.885, 3.370, 3.487, 3.900, 4.067, 4.191, 5.207, 6.724 (Stigmasterol), 17.698 (Lupeol), 18.174 (β-Sitosterol), 23.118, whereas in Dashmool peaks at retention time 2.888, 2.971, 3.135, 3.442, 4.018, 4.220, 4.406, 4.885, 5.388, 5.657, 6.083 (Stigmasterol), 7.848, 9.137, 10.012, 17.656 (Lupeol), 18.138 (β-Sitosterol), 23.096. These peaks showed that there are different compounds and characteristic fingerprints for each drug to judge in herbal formulations. There normalized fingerprints are principal markers that can check the purity/impurity of drug at very low concentration.
Antimicrobial Activity:

Therefore, in present investigation, attempts were made to screen various bioefficacies, to scientifically validate the selected plant in terms of markers. It is, therefore, necessary to standardize the medicinal plants widely used throughout the world. In view of the current importance of interest in herbal drugs, it is necessary to prepare an International Codex containing the details of such plants so that their sale and utilization could be controlled judiciously. The prime objective of this work is to study and set up certain fundamental diagnostic standards for the identification and authentication of G. sylvestre (gudmar), A. lunulatum (Hasraj), B. laciniosa (shivlingi), T. grandis (sagwan), V. odorata (banpasha), Dashmool, S. xanthocarpum (pasarkateli), W. coagulans (paneerphal) used in the Ayurvedic system of medicine.

Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extracts on bacteria and fungi have been studied by a very large number of researchers in different parts of the world. Much work has been done on ethno medicinal plants in India. Interest in a large number of traditional natural products has increased.

The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products. In the present work, a few selected medicinal flora were screened for potential antimicrobial activity by disc diffusion method and found significant results. G.sylvestre (gudmar) petroleum ether extracts showed the best activity against C. albicans (24.6 mm) at than other extracts, A. lunulatum (hansraj) methanol extract showed the highest inhibition zone against K.pneumoniae (35.33 mm), B. laciniosa plant part fruit and stem were screened as antimicrobial agent and fruit methanol extract showed the maximum efficacy against C. albicans (23.3 mm) and stem showed the maximum efficacy against C. albicans (26 mm).

It showed that the B. laciniosa plant is the highly effective against C. albicans fungal infection and also inhibits the growth of other microbial infections. T. grandis (sagwan), leaf and stem was also screened for antimicrobial efficacy against selected test microorganisms. Methanol extract of T.grandis (sagwan) leaf showed maximum inhibition zone against 21.33 mm and stem extract against S. flexneri (24 mm). V. odorata (banpasha) methanol extract is highly effective against S. aureus 21.33 mm and 21 mm at
different concentrations. Dashmool is the combination of 10 Indian medicinal plants, its ethyl acetate extract showed the maximum inhibition zone against *S. flexneri* (25 mm), *S. xanthocarpum* (pasarkateli) methanol extract is highly effective against *S. aureus* 23 mm and *W. coagulans* (paneerphal) ethyl acetate extract inhibit the growth of *E. aerogenes* (28 mm). The serial dilution assay suggested that the inhibitory effects of the 6 extract-bacteria and fungi combinations were dose dependent. In all cases, concentration explained a significant amount of variation in the proportion of bacteria and fungi killed as measured using the statistical analysis and graphs showed a graphical example of the statistical relationships.

Many medicinal plants have been found effective in the cure of bacterial disease. Petroleum ether, benzene, chloroform, ethyl acetate, ethanol and distilled water extracts of certain Indian Medicinal Plants *G. sylvestre* (gudmar), *A. lunulatum* (Hasraj), *B. laciniosa* (shivlingi), *T.grandis* (sagwan), *V. odorata* (banpasha), Dashmool, *S. xanthocarpum* (pasarkateli), *W. coagulans* (paneerphal) were examined for their anti-microbial potentials against selected bacteria and fungi. The purpose of screening is to justify, authenticate and validate the use of Indian Medicinal Plants in ethno-medicinal or folklore as traditional treasure to cure various ailments and disease caused by environmental pollution. In present investigations attempts were made to screen the Indian Medicinal Plants as antibiotics. The various extracts from traditional medicinal plants with folklore reputation have been examined to identify the source of therapeutic drugs and were tested against selected test bacteria and fungi as antimicrobial assay through disc diffusion assay where standard tetracycline is used. Indian Medicinal Plants have a traditional background that they have potentials to use as antimicrobial agents. The results showed that all the extracts possess good antimicrobial activity against selected test bacteria and intermediate against fungus. They offer a scientific basis for traditional use of petroleum ether, benzene, chloroform, ethyl acetate, ethanol and distilled water extracts of *G. sylvestre* (gudmar), *A. lunulatum* (Hasraj), *B. laciniosa* (shivlingi), *T. grandis* (sagwan), *V. odorata* (banpasha), Dashmool, *S. xanthocarpum* (pasarkateli), *W. coagulans* (paneerphal) and thus justified their use in our traditional system of medicine to cure various diseases.

During MIC determination, the lowest MICs were obtained of the plants, indicating that the microbial strains were more sensitive to the extracts. No correlation was noted between the antibiotic susceptibility of the strains and their susceptibility to the plants, as the
plants effectively inhibited antibiotic resistant strains, while some antibiotic sensitive strains appeared to show resistance to the plants.

Fig. 5.1. Showing the % extractive of *G. sylvestre* in serial solvents.

Fig. 5.2. Showing the % extractive of *A. lunulatum* in serial solvents.

Fig. 5.3. Showing the % extractive of *B. laciniosa* (fruit) in serial solvents

Fig. 5.4. Showing the % extractive of *B. laciniosa* (stem) in serial solvents

Fig. 5.5. Showing the % extractive of *T. grandis* (leaf) in serial solvents

Fig. 5.6. Showing the % extractive of *T. grandis* (stem) in serial solvents

Fig. 5.7. Showing the % extractive of Dashmool in serial solvents

Fig. 5.8. Showing the % extractive of *V. odorata* in serial solvents

Fig. 5.9. Showing the % extractive of *S. xanthocarpum* in serial solvent

Fig. 5.10. Showing the % extractive of *W. coagulans* in serial solvent
Under present study, the extractive percent of 8 Indian medicinal plants in 6 different solvents were observed, in which *B. laciniosa* fruit showed the presence of high amount of steroids. *T. grandis* (stem) showed the presence of high amount of terpenoids. *V. odorata*, Dashmool and *W. coagulans* showed the presence of high amount of phenolics. *T. grandis* (stem and leaf) and *G. sylvestre* showed the presence of high amount of alkaloids. *A. lunulatum*, shivlingi (fruit and stem) and *W. coagulans* showed the presence of glycosides in higher amount as compared to others. The occurrence of these compounds will promote a drug for treatment of various skin diseases. Further work on isolation of the bioactive will escort to the production of antimicrobial drugs from Indian medicinal plants.

**Isolation of Bioactive Compounds**

The NMR spectroscopy and its spectral pattern is highly sensitive and has the capacity to differentiate not only the compound but also the extracts of different plant materials of common identity like genuine and their sample. In brief, thus, a “Monograph” may be concluded from the significant findings of the present study. Besides this, the resulting conclusions are of applied nature. Earlier, microscopic markers were in use to check the genuine sample but, from the present findings newer markers such as fluorescence, TLC fingerprints, HPLC chromatograms and antimicrobial techniques have been generated for their use to separate out all the types of genuine herbals. It is noteworthy that some of these new biological markers (antimicrobial) will further check the shelf life, drug potentials, efficacy and effectivity of the herbals. In present investigations, attempts were made to isolate various pure bio actives from Indian Medicinal Plants. It is noteworthy that β sitosterol, stigmasterol and lupeol were isolated which have potentials source as anti HIV agents. Thus, these plants have potential role in future as drug or therapeutic targets.

**Future use of Medicinal Plants as Antibiotics**

This study therefore provides a basis to the folkloric use of these plants as a remedy for skin disease and other infections caused by the pathogens the world over. It also justifies the folklore medicinal uses and claims about the therapeutic values of these plants as curative agents and we, therefore, suggest further that the purification and characterization of the phytochemicals should be obtained with a view to obtain useful chemotherapeutic agents.

In our present research work, it was noted that *V. odorata* (banpasha) and dashmool have potential antibiotic activity more than standards as a future source of drug than other
medicinal plants selected during my research work. MIC values also showed their potentials as antibiotics. Therefore, these ayurvedic preparations can work as therapeutic targets in future. Even the synergistic role of various drugs in a therapy is more important than their individual target. Besides, these are not toxic and play an important role in ayurveda for longevity since ages. Now days, use of herbals to cure various diseases is an urgent need to prove their efficacy as antibiotics for future generations. Not only to prove their efficacy as antibiotics but also usefulness against mdr (Multiple Drug Resistant) for curing various micro-organisms are which resistant to other drug.

Further, more or less all the selected Indian Medicinal Plants have also possessed antimicrobial potentials against all test bacteria and fungi which explains that their use in daily life will generate a resistant or immunity to fight against microorganisms. Methanolic extracts of certain Indian Medicinal Plants showed promising antimicrobial potentials against selected test bacteria and fungi. The main aim of these studies is to validate and authenticate the antimicrobial potentials of certain plants and simultaneously, justify their use in the daily diet to cure mankind from certain ailments.