6.1. INTRODUCTION

In recent times, environment and climate change has turned out to be a subject of rising attention to microbiologists, clinicians, epidemiologists, and ecologists. The research community is highly concerned with the increasing problems of antibiotic resistance in the light of pathogens, emergence and/or re-emergence of infectious diseases.

Environmental degradation exerts extreme pressure on human health. Exposure to air, water and soil pollution, to chemicals in the environment, and to noise, can cause cancer, respiratory, cardiovascular and communicable diseases, in addition to poisoning and neuropsychiatric disorders.

Environment protection is a central element of any sustainable comprehensive growth strategy. This feature of development is particularly significant when awareness of the dangers of environmental degradation has highly increased. Population growth, urbanization, and anthropogenic development employing energy-intensive technologies have resulted in injecting dangerous pollutants into the environment.

“About 40 percent of deaths in the world are caused by water, air and soil pollution”, concludes a Cornell researcher. Such environmental degradation is the main cause behind the rapid raise in human diseases, which the World Health Organization has recently reported. Both factors add to the malnourishment and disease susceptibility of 3.7 billion people. From the environmental assessment made, it was concluded that the dumpsite exposes the residents around it to unacceptable levels of environmental pollutants with unfavourable health impacts. A large number of children and young people living around the dumping site had illnesses linked with respiratory, gastrointestinal and dermatological systems such as upper respiratory tract infections, chronic bronchitis, asthma, fungal infections, allergic and unspecified dermatitis/purities – inflammation and itchiness of the skin.

Simultaneously, more microbes are becoming more and more drug-resistant, and the global warming, together with changes in biological multiplicity, influence parasite evolution and the capability of exotic species to attack new areas. Microbial diseases of the skin are frequently transmitted by being in touch with an infected individual. Although skin in general provides a barrier to infection when it is penetrated by microorganisms, infection develops. Diseases of the eye are measured with the skin diseases because both happen at the exterior of the body.
Skin is the most sensitive part of human body. Pollution leads to diverse types of skin disorders. Air pollution can be a major cause of pre-aging of skin. Dust and mist might generate eczema if in contact with skin. Chemicals used in various products (paints, cleaning stuffs, lacquers, adhesives, building materials etc.) can critically pollute the air. Those agents enter into our body along with breathing, and affect our lungs, eyes, nose, and can generate skin allergies. Ozone is the basic constituent in smog which leads to skin cancer. Besides ozone, the other reason of skin cancer is UV radiation of sun.

Heavy metals, which are usually present in groundwater in various areas, are also harmful to health. In general, urban area drinking water gets contaminated when leaky water pipe joints and sewage line pass close together. Halogen acne, chemical de-pigmentation, connective tissue diseases and skin cancer are some general skin diseases that are caused due to pollution.

The medicinal plants are used in pharmaceutical, cosmetic, agricultural and food industry. The use of the medicinal herbs for curative disease has been recognized in history of all civilizations. Man in the pre-historic era was most likely not attentive to the health hazards related with irrational therapy. With the start of research in medicine, it was concluded that plants have active potency for healing.

Plants and plant extracts have been used for the treatment of skin disorders for centuries. Because of increasing resistance to antibiotics of many bacteria, plant extracts are of new interest as antiseptics and antimicrobial agents. Staphylococcal skin infections are common because they are nearly always present on the skin. Most bacterial infections can cause skin diseases as skin is a haven for many microbes. The skin is exposed to a broad variety of biological, chemical and physical attacks. Among them is solar ultraviolet (UV) radiation and following UV exposure, reactive oxygen species (ROS) are produced and are believed to be largely responsible for skin damage which includes erythematic, photo ageing and cancer. Modern techniques such as HPTLC, HPLC, and TLC etc. can be used to develop the methods for the quantification of marker compounds in these types of multi-component herbal formulations.

Medicinal plants symbolize a rich source of antimicrobial agents. Plants are used medicinally in different countries, and are a basis of many strong and powerful drugs
A broad range of medicinal plant parts are used for extract as uncooked drugs and they have varied medicinal properties. The different parts used include root, stem, flower, fruit, and twigs and modified plant organs. While some of these raw drugs are consumed in lesser quantities by the local communities and folk healers for local use, many other raw drugs are consumed in larger quantities and traded in the marketplace as raw substance for many herbal industries (Uniyal, 2006). Even though hundreds of plant species have been used for antimicrobial properties, the enormous majority have not been sufficiently evaluated (Balandrin, 1985). Considering the huge potentiality of plants as sources for antimicrobial drugs with orientation to antibacterial and antifungal agents, a systematic investigation was undertaken to screen the local flora for antibacterial and antifungal activity from 8 Indian medicinal plants, *G. sylvestre* (gudmar)-Whole plant, *A. lunulatum* (Hasraj)-Whole plant, *B. laciniosa* (shivlingi)-Fruit and stem *T. grandis* (sagwan)-Stem and leaf, *V. odorata* (banpasha)-Whole plant, Dashmool, *S. xanthocarpum* (pasarkateli)-Whole plant, *W. coagulans* (paneerphal)-Fruit.

6.2. REVIEW OF LITERATURE

Discovery of curative powers in plants is an ancient practice. People on all continents have extensive practical poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants, dating back to prehistory. Our ancient literature can also be tapped for information on medicinal plants. No authentic record of any kind except a few archaeological sculptures of Mohenjo-Daro available from the pre-vedic period in this country. But, Rigveda and Atharvaveda, which date back to 2000 to 1000 B.C., contain valuable information regarding medicinal plants of that period. Sharma (1968-69) enlisted 248 botanical drugs which are mentioned mainly in Atharvaveda and Rigveda. Approximately, there are 250,000 to 500,000 species of plants on Earth (Borris, 1996). Relatively small percentages (1 to 10%) of these are used as foods by both humans and other animal species. It is probable that yet more are used for medicinal purposes (Moerman, 1996).

It was decided that extracts of plants would be used fresh and not stored, as there is a possibility of loss of activity of certain extracts after cold storage. This is apparently due to chemical modification of active components or to their precipitation over time (Eloff, 1999). Discussions with a local traditional healer (K. Tucker), confirmed this decision, and it was also suggested the plants be used fresh as opposed to dry, as many of the traditional healers in general, prefer fresh plants and much of the activity is lost if dried. Various methods have

A broad sheet of microbial pathogens linked with a diversity of skin infections has been prepared as shown: The Gram-positive Staphylococcae and Streptococcae are causing wound infections, furuncles, carbuncles, abscesses, impetigo and erysipelas (Kohler, et al., 2001, Madigan, et al., 2003). Gram-negative Enterobacteria are fraction of the physiological intestinal flora. They may cause wound infections and sepsis on the exterior intestine (Kohler, et al., 2001, Madigan, et al., 2003). Pseudomonas, one more Gram-negative rod, is a common pathogen of wound infections. Anaerobic Gramnegative rods may cause skin infections under certain conditions, i.e. in immunocompromised subjects (Kohler, et al., 2001, Madigan, et al., 2003). Grampositive Corynebacteria and Propionibacteria are part of the physiological skin flora. However, Corynebacteria may cause opportunistic skin infections in immunosuppressed patients. Propionibacterium acnes play an important role as causative agent in acne vulgaris (Kohler, et al., 2001). The yeast- C. albicans and C. krusei may occur in low frequency on skin and mucous membranes without causing symptoms. As opportunistic pathogens they may overgrow the normal skin flora and cause skin diseases like intertrigo and candidiasis in diabetics, adipose and immunodeficient subjects. The dimorphic yeast Malassezia furfur that is growing in skin areas rich in sebaceous glands is associated with the pathogenesis of seborrhoic eczema and dundruff (Faergemann, 2004 and Grigoriu, et al., 1984).

Mainstream medicine is ever more receptive to the use of antimicrobial and other drugs derived from plants, as conventional antibiotics (products of microorganisms or their synthesized derivatives). One heavier factor for the renewed interest in plant antimicrobials in the past 20 years has been the fast rate of (plant) species extinction (Lewis and Elvin-Lewis, 1995).

6.3. MATERIALS AND METHODS

6.3.1. Collection

Plant samples of 8 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 procured from various tribes living in tribal pockets of Mt. Abu, arid zone of Rajasthan, in the month of Feb, 2010. These plants were used by these tribes in their daily lives to cure various ailments.
6.3.2. Identification

All the samples were authenticated and were given identification number.
After authentication, these samples were submitted to Ethnomedicinal Herbarium, Centre of Excellence (funded by DST), MGIAS, Jaipur (Rajasthan).

6.3.3. Processing of plant materials

During the course of the study, each sample was screened for its foreign matter and milled before use.

6.3.4. Experimental details

Present studies were performed on 8 Indian medicinal plants for the following activities:-
1. Extraction
2. Phytochemical test of plant extracts.
3. TLC and HPLC
5. MIC
6. NMR spectrum of isolation pure compounds.
7. Statistical analysis

6.3.4.1. Extraction

Selected powdered drug (g) was refluxed with petroleum ether, chloroform, benzene, ethyl acetate and methanol and distilled water for 6 hours, filtered, concentrated to dryness and was used for chromatography. Simultaneously each drug (g) was soxhlet extracted successively with petroleum ether, chloroform, benzene, ethyl acetate and methanol and distilled water in for 18 hours. These extracts were filtered, evaporated to dryness and weighed for antimicrobial screening.

6.3.4.2. Phytochemical Screening

Phytochemical screening was performed using a standard procedure:
1. Test for reducing sugars (Fehlings test)
2. Test for terpenoids (Salkowski test)
3. Test for flavonoides
4. Test for tannins
5. Test for saponins
6.3.4.3. TLC (Thin Layer Chromatography) and HPLC (High Pressure Liquid Chromatography)

Concentrated extracts were used for isolation and characterization of compounds. Crystallization by the solvent Hexane: Acetone (3:1) was performed to separate the pure bioactives which was checked by thin layer chromatography and high pressure liquid chromatography (Harborne, 1973). Also, various Indian medicinal plants were subjected onto the HPLC analysis using Shimadzu Model LC2010 AHT Auto Sampler (UV-VIS Detector).

6.3.4.4. Antimicrobial Activity

In present investigations, attempts were made to screen selected 8 Indian medicinal plants as potent antimicrobial agents to cure the future generation from infectious skin diseases. Further, these extracts could also be used as herbal drugs. Various extracts/secondary metabolites rich fractions/bioactives were screened for various bioactivities i.e. antimicrobial – antibacterial and antifungal by disc diffusion method (Gould and Bowie, 1952).

6.3.4.5. MIC value (Minimum inhibitory concentration)

Antimicrobial activity against namely P. aeruginosa, S. aureus, K. pneumoniae, S. typhi, S. flexneri, P. vulgaris, E. aerogenes and fungi C. albicans, A. niger, T. rubrum microbes was inferred through replicate disc diffusion assays; and observed and statistically predicted MIC values were determined through replicate serial dilution assays. Minimum inhibitory concentration (MIC) was determined for each plant extract showing antimicrobial activity against test microorganisms. At the end of incubation, the plates were collected and zones of inhibition that developed were measured. The average of the zones of inhibition was calculated. The minimum inhibitory concentration (MIC) was calculated by plotting the natural logarithm of the concentration of extract against the square of zones of inhibition. A regression line was drawn through the points. The antilogarithm of the intercept on the logarithm of concentration axis gave the MIC values (Esimone, et al., 1998, Osadebe and Ukwueze, 2004).

6.3.4.6. NMR spectrum of isolation pure compounds

The compound was subjected to NMR analysis (model Brukur-DPX-300 MHz, using CDCl₃ and DMSO- d₆ as an internal reference) along with the standard reference compound.

6.3.4.7. Statistical analysis
All the data were analysed by standard statistical analysis methods.

**OBJECTIVES**

1. Collection
2. Identification
3. Processing of plant material
4. Experimental details
   a) Phytochemistry
   b) TLC
   c) HPLC
5. Specific objective
   a) Selection of plants.
   b) Phytochemistry characterization of active extracts.
   c) Extraction and isolation of active extract/fraction/bioactive as antimicrobial agents.
   d) TLC for characterization of active extracts.
   e) HPLC (qualitative and quantitative studies)
   f) NMR spectrum of isolation pure compounds.
   g) Antimicrobial assay against selected bacteria.
   h) MIC (Minimum Inhibitory Concentration)

6.4. RESULTS AND DISCUSSION

6.4.1. Extraction
   For experimental profile of selected species, each of the dried and powdered (g) test samples was soxhelt extracted in petroleum ether, benzene, chloroform, ethyl acetate, methanol and distilled water for 6 hours. These extracts were filtered, evaporated to dryness and weighed. Each extract was used for phytochemical, TLC and antimicrobial screening of plant active compound.

6.4.2. Phytochemical screening
   In the present study, (G. sylvestre (gudmar), A. lunulatum (Hasraj), B. laciniosa (shivlingi), T. grandis (sagwan), V. odorata (banpasha), Dashmool, S. xanthocarpum (pasarkateli), W. coagulans (paneerphal) medicinal plants were phytochemically evaluated for various tests i.e. reducing sugar, saponins, tannins, flavonoids and terpenoids and discovered appreciable results.

6.4.3. Thin Layer Chromatography (TLC) and HPLC (High Pressure Liquid Chromatography)
   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. The resultant differential chromatographic “fingerprints” can actually be used as “markers” in the standardization of each extract in a particular solvent system separating the compounds at specific Rf value, which will differ from other plant extracts. These Rf values are simple, reproducible and thus
reliable markers 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* (hansraj), *B. laciniosa* (stem), *T. grandis* (leaf), *T. grandis* (sagwan, stem), *V. odorata* (banpasha) and Dashmool using solvent system Hexane: Acetone (3:1). 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 -0.63 (Purple) and lupeol at R_f -0.72 (Pink), *A. lunulatum* showed the presence of β-Sitosterol at R_f -0.60 (Purple) and Lupeol at R_f -0.63 (Pink), *B. laciniosa* (stem) showed the presence of Stigmasterol R_f -0.63 (Purple) and lupeol at R_f -0.72 (Brown), *T. grandis* (leaf) showed the presence of β-Sitosterol R_f -0.56 (Purple), Stigmasterol R_f -0.58 (Purple) and Lupeol at R_f -0.64 (Pink), *T. grandis* (sagwan, stem) showed the presence of β-Sitosterol R_f -0.55 (Pink), Stigmasterol R_f -0.62 (Purple) and Lupeol at R_f -0.66 (Purple), *V. odorata* (banpasha) showed the presence of β-Sitosterol R_f -0.55 (Pink), Stigmasterol R_f -0.58 (Purple) and Lupeol at R_f -0.60 (Purple), whereas Dashmool showed the presence of Lupeol at R_f -0.72 (Purple), Stigmasterol at R_f -0.62 (Purple) and β-Sitosterol at R_f -0.60 (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 and 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 in analytical and semi preparative mode under 254 nm, the retention time recorded at 18.138, 6.714 and 17.656, which showed that as the column size increases, it affects on retention time (column size α retention time). It also affects the peak sharpness.

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 were different compounds and characteristic fingerprints for each drug to judge in herbal formulation. These normalized fingerprints were principal markers that could check the purity/impurity of drug at very low concentration.

6.4.4. Antimicrobial activity

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 floras were screened for potential antimicrobial activity by disc diffusion method and found significant results as listed below:

1. \textit{G. sylvestre} (gudmar) petroleum ether extracts showed the best activity against \textit{C. albicans} (24.6 mm) at different concentration than other extracts.

2. \textit{A. lunulatum} (hansraj) methanol extract exhibited the highest inhibition zone against \textit{K. pneumoniae} (35.33 mm).

3. \textit{B. laciniosa} plant part fruit and stem were screened as antimicrobial agent and fruit methanol extract showed the maximum efficacy against \textit{C. albicans} (23.3 mm) and stem showed the maximum efficacy against \textit{C. albicans} (26 mm). It also revealed that the \textit{B. laciniosa} plant is highly effective against \textit{C. albicans} fungal infection and also inhibits the growth of other microbial infections.

4. \textit{T. grandis} (sagwan), leaf and stem were also screened for antimicrobial efficacy against selected test microorganisms. Methanol extract of \textit{T. grandis} (sagwan) leaf demonstrated maximum inhibition zone against 21.33 mm and stem extract against \textit{S. flexneri} (24 mm).

5. \textit{V. odorata} (banpasha) methanol extract is highly effective against \textit{S. aureus} 21.33 mm and 21 mm at different concentrations.
6. Dashmool is the combination of 10 Indian medicinal plants roots, its ethyl acetate extract revealed the maximum inhibition zone against \textit{S. flexneri} (25 mm).

7. \textit{S. xanthocarpum} (pasarkateli) methanol extract is highly effective against \textit{S. aureus} 23 mm.

8. \textit{W. coagulans} (paneerphal) ethyl acetate extract maximum inhibit the growth of \textit{E. aerogenes} (28 mm).

The serial dilution assay suggested inhibitory effects of the 6 extract-bacteria and fungi combinations and revealed that the disc diffusion assay were dose dependent. In all cases, concentration explained a significant amount of variation in the proportion of bacteria and fungi killed as measured and analysed statistically. The graphs show statistical relationships.

6.4.5. MIC (minimum inhibitory concentration)

During MIC determination, the lowest MICs were obtained from 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.

6.4.6. NMR spectrum of isolation pure compounds

The NMR spectroscopy and its spectral outline is extremely responsive and has the ability to distinguish not just the compound but also the extracts of different plant materials of common individuality like genuine and their adulterants sample.

In brief, thus, “Monograph” may be completed from the important findings of the current study. Besides this, the resultant conclusions are of practical nature. Earlier, phytochemical analysis were in use to check the sample but, from the present findings newer techniques such as TLC fingerprints and antimicrobial techniques have been generated for their use to separate out the potential ability of a drug to work against skin disease and provide a tool for curing the disease as infections grow increasingly and hence offer a source as antimicrobial agents to prove as a anti-microbial herbal source for therapeutics. It is noteworthy that some of these new biological efficacies (antimicrobial) will further check the shelf life, drug potentials, and effectivity of the herbals.
6.4.7. Statistical analysis

Diameter of bacterial and fungal growth was measured and expressed as means of percentage growth inhibition of three replicates. Significant differences within the means of treatments and controls were calculated using statistical test.

The MIC is defined as the lowest concentration of antibiotic or extract at which there is no visible growth. The agar plates without extract or standard antibiotic (the negative control) and the plates containing standard antibiotics, tetracycline (the positive control) were also streaked with the micro organisms. The agar plates were incubated at 37°C for 24 hours (for the bacteria) and at 25°C for 48 hours (for the fungus). The inhibition zone diameter, the measure of activity, was consequently determined by plotting the square of the inhibition zone diameter (IZD2) against the log concentration of the extract and the MIC calculated from the intercept on the log concentration axis.

6.5. CONCLUSION

Many medicinal plants have been found effective in the cure of bacterial disease. Petroleum ether, benzene, chloroform, ethyl acetate, methanol 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. At present, 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; they 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 possessed good antimicrobial activity against selected test bacteria and intermediate against fungus, therefore offer a scientific basis for traditional use of petroleum ether, benzene, chloroform, ethyl acetate, methanol 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), it justified their use in our traditional system of medicine to cure various diseases.
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 microorganisms are which resistant to other drugs.

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.