CHAPTER 1

INTRODUCTION

Plants have been the source of treatment for many ailments since ancient times. In developing countries as in India, 25% of the medical drugs are based on plants. The use of their derivatives is well known among the indigenous people in rural areas of many developing countries. The National Health Services (2003) have reported that historically all medicinal preparations were derived from plants, whether in the simple form of plant parts or in the more complex form of crude extracts and mixtures. Ayyanar and Ignacimuthu (2009) have reported that several drugs of plant, mineral, and animal origin are described in the Ayurveda for their effective treatment of many diseases like jaundice, pneumonia, malaria and gastrointestinal problems. The wound healing properties of many flora and fauna in Ayurveda has been described under the term "Vranaropaka". In the traditional systems of medicine, various plants extracts have been used to promote the wound healing activity for cuts and burn wounds by healing in the natural way.

1.1 Etiology of wound infections

Wound infection is one of the most common nosocomical infections in developing countries usually occurring due to poor hygienic conditions (Senthil et al., 2006; Meenakshi et al., 2006; Enoch et al., 2005 and Clavin et al., 1998). Wounds are the physical injuries that results in an opening or breaking of the skin and appropriate method for healing of wounds is essential for the restoration of disrupted anatomical continuity and epithelial integrity of the skin. Wounds are injuries to body tissues caused by physical trauma or disease processes that may include surgery, diabetes, burns, punctures, gunshots, lacerations, bites, bed sores, and broken bones. Types of wounds may include:
- Abraded or abrasion: Caused by scraping, such as falling on concrete.
- Contused or contusion: A bruise or bleeding into the tissue.
- Incised or incision: A wound formed by a clean cut, as by a sharp instrument like a knife.
- Lacerated or laceration: A wound caused by heavy pressure, causing tearing of the skin or other tissues.
- Nonpenetrating: An injury caused without disruption of the surface of the body. These wounds are usually in the thorax or abdomen and can also be termed blunt trauma wounds.
- Open: A wound in which tissues are exposed to the air.
- Penetrating: Disruption of the body surface and extension into the underlying tissue.
- Perforating: A wound with an exit and an entry, such as a gunshot wound, and,
- Puncture: A wound formed when something goes through the skin and into the body tissues. This wound has a very small opening, but can be very deep in nature.

Wounds are generally classified as, wounds without tissue loss (as in surgery) and wounds with tissue loss, such as burn wounds. Healing of wounds starts from the moment of injury and can continue for varying periods of time depending on the extent of wounding and the process can be broadly categorized into three stages: inflammatory phase, proliferate phase and finally the remodeling phase which ultimately determines the strength and appearance of the healed tissue. These phases are concurrent but independent of each other. The development of a wound infection depends on the complex interplay of many factors such as state of hydration, nutrition and existing medical conditions as well as extrinsic factors such as those related to pre-, intra-, and post-operative care if the patient has undergone surgery. The healing process can be physically monitored by assessing the rate of contraction of the wound, period of epithelization, tensile strength, histopathology, and
weight of granuloma in different wound models (Ayton, 1985). According to Bowler (1998), if the integrity and protective function of the skin is breached, large quantities of different cell types will enter the wound and initiate an inflammatory response which aims to restore homeostasis.

1.2 Pathogenicity and complexity of wound infections

In surveillance by NINSS in 2002, the presence of a micro-organism within the margins of a wound does not indicate that wound infection is inevitable. In case of surgical wounds and infection in wounds due to accidents, a number of specific factors have also been identified in relation to infection rates (Flanagan, 1997). These include:

- Presence of an existing chronic infection.
- Time interval between skin preparation and surgery.
- Nature of the invasive procedure - especially if involving the bowel.
- Extent of tissue loss and/or trauma to tissues during surgery.
- Adequacy of wound drainage.
- Appropriate use of wound management materials.

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Table 1.1: Examples of potential wound pathogens
(Courtesy: http://www.worldwidewounds.com/2004/january/)
The worldwide emergence of antimicrobial resistance among a wide variety of human bacterial and fungal burn wound pathogens, particularly in nosocomial isolates, limits the available therapeutic options for effective treatment of burn wound infections. Prior to the antibiotic era, *Streptococcus pyogenes* (group A beta-hemolytic Streptococci) was the predominant pathogen implicated in burn wound infections and was a major cause of death in severely burned patients. But shortly, after the introduction of penicillin G in the early 1950s, it resulted in the virtual elimination of *S. pyogenes* as a cause of infection in thermally injured patients.

Today with the rise in the level of treatment, resistance within the pathogenic microbes has also become elevated in such a way that MRSA, methicillin-resistant coagulase-negative Staphylococci, vancomycin-resistant Enterococci and multiple resistant Gram-negative bacteria that possess several types of beta-lactamases have emerged as serious pathogens in hospitalized patients. A study by Ekrami & Kalanter (2007) highlighted the high levels of antimicrobial resistance in *Pseudomonas aeruginosa* while fifty eight per cent of *S. aureus* and sixty percent of coagulase negative Staphylococci were methicillin resistant. In a prospective study conducted by Sharma and Taneja (2004) at the Burns unit at Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, *S. aureus* and *P. aeruginosa* were the most frequent organisms causing wound as well as blood stream infection. Ninety per cent of *P. aeruginosa* were resistant to amikacin and ceftazidime, forty-five percent to ciprofloxacin and twenty-five percent to piperacillin while forty-three percent *S. aureus* were MRSA. Treatment of *Pseudomonas* wound infections, along with the frequently associated shock and respiratory failure, can involve complex fluid and ventilator management, definitive surgical debridement, and advanced wound closure technologies. The ability to accurately follow this bacterial pathogenesis is an important prerequisite for the development of antimicrobial agents.
aimed at controlling the course of infection (Sharma and Taneja, 2007). Pharmacological targeting of nonessential functions such as virulence factor production may decrease the emergence of resistance of the microorganisms against the subjected antibiotics.

1.3 Plants as natural wound healers

The medicinal value of a plant lies in its bioactive constituents and any phytochemical investigations of a given plant reveals only a narrow spectrum of its constituents. These chemical entities derived from plants needs to be identified and formulated for treatment and management of many diseases with required modification as also stated by Fabricant and Farnsworth (2001). Some of these plants have been screened scientifically for the evaluation of their wound healing properties in different pharmacological and experimental models, but the potentiality of most still remains unexplored. Antimicrobial and phytochemical screening has as such become crucial for obtaining these biodynamic compounds as only a small percentage has been phytochemically investigated and the fraction submitted to biological or pharmacological screening is even smaller. In a few cases, active chemical constituents were identified and used for preparing medical formulations. Formulations derived from plants have also found to be effective in experimental models (Edeoga et al., 2005). Many of them act as synergistics and enhance the bioactivity of other compounds (Sumitra et al., 2005). Continuing discovery has also been facilitated by the recent development of new bioassay methods. Active lead compounds can also be further modified to enhance the biological profiles and develop as clinical trial candidates for better drugs.

1.4 In-silico screening of bioactive compounds as potential drug source

According to Ahmed and Aquil (2007) and Cohen (1992), natural products of higher plants may possess new sources of antimicrobial agents with possibly novel mechanisms of
action. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents. A systematic screening of them may result in the discovery of novel active compounds serving as a potential source of biodynamic compounds of therapeutic value in phytochemical research.

*In-silico* screening involves the analysis of physicochemical properties and binding and inhibitory activity of the bioactive compounds, synthetic or natural, within the active site of the selected disease target. Numerous methods have been utilized to acquire and isolate compounds from plants and other natural sources for drug discovery, including synthetic chemistry, combinatorial chemistry and molecular modelling (Iwu et al., 1999 and Evans et al., 2002). Despite the recent interest in these methods by pharmaceutical companies and funding organizations, apart from their use in Ayurveda, natural products, particularly those from medicinal plants, still remains an important but not much explored source of new drugs, new drug leads and new chemical entities (NCEs). Singh and Barrett (2006) reported that of the major classes of antibiotics, all except three are natural products-based, most of them being microbial origin. While the use of natural product discovery platforms yielded many important chemotherapeutics during the mid- and late twentieth century (with natural products accounting for more than 80% of all drugs discovered prior to 1990 as stated by Li and Vederas, 2009), a dramatic shift in the drug discovery paradigm occurred approximately twenty-five years ago (Newman et al., 2003). The introduction of combinatorial chemistry to generate large libraries of small molecules and the advent of high-throughput screening (HTS) technologies eventually replaced many natural product discovery programs (Lee and Breitenbucher 2003). It can now be assumed
that the chemical diversity of secondary plant metabolites that results from plant evolution might become equal or superior to the compounds found in synthetic combinatorial chemical libraries in future.

Specific binding interactions are central to many biological processes and pathways. Similarly, most drugs act by binding specifically to a site on a target protein, thereby modulating protein activity. The quest for new drugs relies on many approaches, including computer-based virtual screening and docking. Over the past fifteen years, and in parallel with the exponential increase in the number of available high-resolution protein structures, many screening and docking methods and programs have emerged for use in the drug discovery process.

Virtual screening is used to discover new drug candidates from different chemical scaffolds by searching commercial, public, or private 3-dimensional chemical structure databases. It is intended to reduce the size of chemical space thereby allowing focus on more promising candidates for lead discovery and optimization. The goal is to enrich set of molecules with desirable properties (active, drug-like, lead-like) and eliminate compounds with undesirable properties (inactive, reactive, toxic, poor ADMET/PK) for creation of a stable compound database. In another words, *in silico* modeling is used to significantly minimize time and resource requirements of chemical synthesis and biological testing.

Virtual compound screening using molecular docking approach is widely used in the discovery of new lead compounds for drug design. Once an optimum library has been produced of the lead molecules, molecules are docked to the target receptor to screen and select the best candidates. This initial screening makes use of fast ranking functions to evaluate the relative stability of the docked complexes. The selected candidates, usually a few hundred, are subjected to further docking experiments using more sophisticated scoring functions. A combinatorial library is thus established which consists of all the valid
and bioactive molecules from the parent source produced through enrichment procedures. Docking the ligands against each protein structure in the ensemble constitutes the most comprehensive, although expensive approach followed by lead optimization and final refinement for production of a reliable combinatorial library.

The main classes of virtual screening methods depend on the amount of structural and bioactivity data available for

1. One active molecule known: through similarity search (ligand-based virtual screening),
2. Several active molecules known: a 3D database search on a common 3D Pharmacophore,
3. Reasonable number of active and inactive structures known, and
4. Three dimensional structure of the protein known using protein-ligand docking.

1.5 Approaches to drug discovery using higher plants

Till today, of the total floral species in the world, only about six percent have been so far estimated for their biological activity and a reported fifteen percent have been evaluated phytochemically (Verpoorte, 2000).

The goals of using plants as sources of therapeutic agents are

a) to isolate bioactive compounds for direct use as drugs, e.g., digoxin, morphine, reserpine, taxol, vinblastine, vincristine;

b) to produce bioactive compounds of novel or known structures as lead compounds for semisynthesis to produce patentable entities of higher activity and/or lower toxicity, e.g., metformin.

According to Khalil et al., (2007) many traditional remedies are based on systematic observations and methodologies and have been time-tested. But for many of them, scientific evidence is lacking and there are only few prospective randomized controlled trials that have proved the clinical efficacy of these traditional wound healing agents. Comprehensive evaluation on the plants with wound healing activity on the basis of traditional medicine may
possibly give new compounds that could be used as prominent drugs in wound healing therapy. Kumar et al., (2007) stated that, earlier, the major problem with pharmacological validation of the wound healing plants was that the exact mechanism of the healing process of wound was not clearly understood; hence most of the researchers restricted the screening of plants to simple healing of wounds externally. Today with the development of improved technology and techniques the validation by scientific method of the usefulness of various plant species can form the basis for their use as alternative treatment or when conventional therapy by Western medicine is unavailable.

1.5.1 Antimicrobial activity of *Garcinia* against wound infections

*Garcinia* is a large genus of polygamous trees or shrubs belonging to the family *Clusiaceae*, distributed in the tropical Asia, Africa and Polynesia and is a rich source of bioactive molecules including xanthones, flavonoids, benzophenones, lactones and phenolic acids. Some of them have shown a wide range of biological activities including antibacterial, antifungal, antioxidant and cytotoxic effects. *Clusiaceae* are a family of approximately 1000 species with a pantropical distribution. According to Suksamrarn et al., (2006) based on a morphology-based cladistic analysis, the family has been divided into three subfamilies: *Hypericoideae*, *Clusioideae*, and *Kielmeyeroideae*.

The genus *Garcinia*, belonging to family *Clusiaceae*, is named after Laurence Garcin who collected and studied these species in the eighteenth century in India (Sahni 1998). Linnaeus named the genus for Laurent Garcin, born at Grenoble, France in 1683.

The genus *Garcinia* is native to Asia, Australia, tropical and southern Africa, and Polynesia. It consists of about 180 species, of which about 30 species are found in India. The *Garcinia* species grows in highest density in the north of the Malaysian Archipelago, while 18 tropical species are found in the Andaman and Nicobar Islands, the North-East, Assam, Tamil Nadu and Western Ghats (Pedraza Chaverri et al., 2008).
Economically the fruit which is the most important plant part is a leathery indehiscent berry, seeds surrounded by pulpy often edible aril. Leaves are lanceolate or elliptic, leathery, petioles with a margined pit on upper side at base. Flowers are small, yellowish green, solitary or the male flowers in axillary clusters (Grabley et al., 1999).

Commercial gamboge is the resin of *G. hanburyi* and *G. morella*. Their application in wound healing has also been reported in many cases. *Garcinia mangostana Linn*, the fruit hulls have been in use in Thai folk medicine for the treatment of fungal infections, inflammation, skin infections, wounds and diarrhea (Grabley and Thiericke, 1999). *Garcinia indica* fruits contain rich amounts of anti-oxidants that bind with free radicals and prevent oxidative damage to body cells (Thomas et al., 2007). *Garcinia indica* also promote cell regeneration and repair. *Garcinia indica* fruit has a soothing and healing property and can be applied directly to wounds and infected areas on the skin. Acid fruits of several species are used in external medicine. Commonly, the plants in this genus are called saptrees. *Garcinia* species are evergreen trees and shrubs, dioecious and in several cases apomictic.

The plants native to North-East India within this genus are

1. *Garcinia cowa* Roxb. ex D.C.(Vern:Kau thekera)
2. *Garcinia lancifolia* Roxb.(Vern : Rupahi Thekera)
3. *Garcinia morella* (Gaertn.) Desr.(Vern : Kuji Thekera )
4. *Garcinia paniculata* Roxb.(Vern : Sochopa Tenga)
5. *Garcinia pedunculata* Roxb.(Vern : Bor thekera)
7. *Garcinia accuminata*
1.6 Need for creation of a database

Ayurveda, Unani, Kampo and traditional Chinese medicine have flourished as systems of medicine in use for thousands of years. Their individual arrangements all emphasize education based on an established, frequently revised body of written knowledge and theory. These systems are still in place today because of their organizational strengths through which they focus primarily on multicomponent mixtures. Even though Western medical science views such systems as lacking credibility, undeniably they are used widely by most people worldwide. Adverse effects from those widely used plants are not well documented in the literature and efficacy of these plants and plant mixtures is more difficult to assess by Western scientific methods. Though plant compounds exhibit enormous structural diversity, only a small proportion of that diversity has been seriously explored for its pharmacological potential so far. By comparison with other areas of pharmaceutical research, however, the screening of natural products has suffered a setback from lack of data in an appropriate format. In particular, electronic information on chemical structures, pharmacological activity, specificity against known molecular targets, and traditional uses of the plants in which such compounds are found has been insufficient in a single database, though, an increasing amount of information has become available in recent years (Sneader, 2005).

In view of all this findings, systematic exploration of medicinal plants used by traditional healers of India particularly in North-East is very important to reveal and characterize the active constituents and subsequently develop a solid database based on all these findings. There are many potential wound healers in North-East India. But no proper records have been maintained as such for these plants based on their wound healing property. It is necessary that documentation of the medicinal plants may be treated as an extreme urgency as changes in land use due to urbanization is destroying much of the habitat of useful
plants leading to irreversible loss of many species. Therefore, a great necessity was felt to have a relevant digital library providing multidisciplinary information on these useful plants, starting with *Garcinia*, at one place in the form of a database.

The various approaches to drug discovery from higher plants to create a database include the following steps-

a. Phytochemical screening of plants,

b. Pre-clinical biological assays in experimental models for verification and validation of the screening,

c. Scientific evaluation and follow-up of ethnomedical uses of plants, and

d. Creation of a computer database based on the collected information using digital languages.

Many databases have been produced worldwide based on various information about the general and medicinal properties of plants based on these traditional information validated with scientific studies. Some databases created so far are like the TTD (bidd.nus.edu.sg/group/cjttd/), EROWID (http://www.erowid.org/), NAPRALERT (www.napralert.org/), USDA-DUKE (www.usda.gov, www.ars-grin.gov/duke/). On November, 2000, the Bio-diversity Documentation Centre under Jawaharlal Nehru Centre for Advanced Scientific Research has created a Digitized Inventory of Plant Resources of India for economically important plants other than medicinal species (http://www.jncasr.ac.in/bdu/). The InPACdb developed by Vetrivel et al., (2009) is an Indian plant anticancer compounds database comprehensive information covering cancer type, molecular target, 3D Stereochemical structures (tautomers, stereoisomers, conformers and resonance structures) and Chemical descriptors etc. for each entry and enabling effective cheminformatics analysis (http://www.inpacdb.org/). The National Medicinal Plant Board (http://nmpb.nic.in/) under Government of India has also developed an online
project monitoring information system with an aim to coordinate all matters relating to medicinal plants and support policies and programmes for growth of trade, export, conservation and cultivation in India. Besides these, some databases in India includes the BGCI plant database (http://www.bgci.org/plant_search.php/) under Botanic Gardens Conservation in India, the Pandanatas database of Medicinal plants (http://iu.ff.cuni.cz/pandanatas/database/), the Encyclopedia on Indian Medicinal Plants (http://envis.frlht.org/indian-medicinal-plants-database.php), the Encyclopedia of Ayurvedic medicinal plants (http://www.indianmedicinalplants.info/plants/) and the Medicinal Plant Database (http://medicinalplantdatabase.com/) among others.

Creation of such biological databases has found its importance for the systematic collection and proper documentation of the various data present across various literatures and libraries around the world so that they can be view and accessed by all. In the drug discovery pipeline, creation of a database of drugs, drug-like compounds, their source and their targets becomes imperative for proper follow-up of their in-vitro, in-vivo and in-silico analysis during or prior to pre-clinical and clinical trials.

With an aim to study the etiology of wounds and wound infection, their inhibition and creation of a database based on all the collected information, the following objectives were taken up for the current research analysis-

The objectives taken up for the undergoing research analysis were,

1. Isolation and identification of microorganisms causing wound infections.
2. Phytochemical and antimicrobial activity analysis of selected species of Garcinia.
3. In-silico drug designing of the possible biologically active compounds identified within the Garcinia species.
4. Selection of protein or disease targets.
5. Ligand protein inverse docking for finding putative protein targets of a small molecule by computer-automated docking search