Introduction
1. INTRODUCTION

Antibiotic resistance is the ability of the microorganism to withstand the effects of an antibiotic. The extensive use of antibiotics over the last 50 years has led to the emergence of bacterial resistance and to the dissemination of resistance genes among pathogenic microorganisms.

The progressive emergence and rapid dissemination of antibiotic resistance in staphylococci and its association with the use and consumption of antibiotic constitute a major health concern and have been considered a global crisis (Chambers, 1997; Martinez and Baquero, 2000). Staphylococci ubiquitous microorganisms present in the respiratory tract and on the skin of a high percentage of adults. However, several population groups are at serious risk of suffering pathogenic staphylococcal infections.

Methicillin-resistant *Staphylococcus aureus* (MRSA) organisms are one among the most important nosocomial pathogens, being responsible for a wide range of infections, some of which are associated with high mortality (Casgrove *et al.*, 2003). In fact, the first MRSA strain was isolated in 1961. Since then the emergence of multi-resistant strains of *S. aureus* carrying resistance to methicillin (MRSA) and to most currently available antibiotics (Boyce, 2001; Hryniewicz, 1999) has dramatically narrowed the therapeutic arsenal to the exclusive use of glycopeptides (such as vancomycin and teicoplanin) as the mainstay of MRSA treatment. Unfortunately, the excessive use of vancomycin has led to the emergence of MRSA strains with decreased susceptibilities to glycopeptides (Hiramatsu, 1998; Park *et al.*, 2000).
MRSA is still considered as an emerging pathogen and public health threats result from the spread of hospital-acquired as well as community-acquired MRSA (Chambers, 2001; Okuma et al., 2002; Oliveira and de Lencastre, 2002). Thus important efforts have been made during the past decades in order to get a detailed knowledge of MRSA epidemiology and to improve infection control strategies (Hiramatsu et al., 2001).

The mechanism of methicillin resistance in *S. aureus* is based on the production of an additional low-affinity penicillin-binding protein (PBP; PBP 2a), which is encoded by the *mecA* gene (Chambers, 1997; Hackbarth and Chambers, 1989; Quintiliani and Courvalin, 1995). Detection of Methicillin-resistant *Staphylococcus aureus* (MRSA) in clinical samples continues to be important, since infections due to MRSA have high morbidity and mortality rates. Moreover, some MRSA strains have the potential to spread rapidly and colonize in other patients (Wertheim et al., 2001). Early detection of MRSA carriers is crucial not only for infection control (Farr and Jarvis, 2002) but also for therapeutic decision with last-line antibiotics against MRSA, e.g., glycopeptides and oxazolidinones (Tsiodras et al., 2001).

*S. aureus* is a versatile human pathogen causing infections ranging from relatively mild involvement of skin and soft tissue to life-threatening sepsis, pneumonia and toxic shock syndrome (TSS) (Chambers, 1997; Tenover and Gaynes, 2000). In the rural communities, wounds arising from bruises, cuts and scratches, amongst others, are sometimes untreated at the initial stages. This is common especially amongst children. In most cases such wounds become septic and inflamed before they are brought to the attention of the parents, who might then treat such wounds in a traditional way using plant materials or seek the advice of herbalists. Even many adults who are remote from clinics and hospitals often treat wounds using plants or seek the help of herbalists. Wounds are injuries to body tissues caused by disease processes or events such as burns, punctures and human or animal bites. The wounds tend to range in size from about 5 to 40 cm². Importantly, the damaged tissue is typically infected by a bacterium which makes the wound more difficult to treat. Wounds or abscesses also occur within body become
infected when microorganisms from the outside environment, or from within the person’s body, enter the open wound and multiply. A wound that is red, painful, swollen and draining pus is probably infected. A fever following surgery indicates an infection at the site of surgery.

Until recently, research and development (R&D) efforts have provided new drugs in time to treat bacteria that became resistant to older antibiotics. The resistance problem demands that a renewed effort be made to seek antibacterial agent effect. One of the possible strategies towards this objective is the rational localization of bioactive phytochemicals. Plants have an almost limitless ability to synthesize aromatic substances, most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects and herbivores. Many of the herbs and spices used by humans to season food yield useful medicinal compounds including those having antibacterial activity (Wallace, 2004; Thuille et al., 2003; Singh et al., 2002).

Many of these plants have been investigated scientifically for antimicrobial activity and a large number of plant products have been shown to inhibit growth of pathogenic bacteria. Though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has developed. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which in turn may be utilized as therapeutic agents (Cohen, 1992). A number of these agents appear to have structures and modes of action that are distinct from those of the antibiotics in current use, suggesting that cross-resistance with agents already in use may be minimal. For example, the combination of 5'-methoxyhydnocarpine and berberine in herbs like Hydrastis canadensis and Berberis vulgaris can block the MDR-pumps that cause multidrug resistance. This has been shown for Staphylococcus aureus (Stermitz et al., 2000).
According to the World Health Organization (WHO), the medicinal plants would be the best source for obtaining a variety of drugs (Santos et al., 1995). For thousands of years, mankind has known about the benefit of drugs from nature. Plant extracts for the treatment of various ailments, were highly regarded by the ancient civilizations (Grabley and Thiericke, 1999). About 80% populations of the developed countries use traditional medicines, derived from medicinal plants. Plant materials remain an important resource for combating illnesses including infectious diseases and many of these plants have been investigated for novel drugs or templates for the development of new therapeutic agents. Plants are also well known to be the risk sources of biologically active compounds. Therefore, one approach that has been used for the discovery of antimicrobial agents from natural sources is based on the evaluation of traditional plant extracts. Aspirin, astropine, ephedrine, digoxin, morphine, quinine, reserpine and tubocurarine are a few examples of drugs, which were originally discovered through the study of traditional cures and folk knowledge of indigenous people. Therefore, such plants should be investigated thoroughly to determine their structural and functional properties, as well as the efficiency of various parts (Ellof, 1998).

There are about 120 plant-based drugs prescribed worldwide, and they come from just 95 plant species. Of the approximately 250,000 species of flowering plants, only 5000 have had their pharmaceutical potential assessed (Lewington, 1990). The treatment of infectious diseases with antimicrobial agents continues to present problems in modern-day-medicine with many studies showing a significant increase in the incidence of bacterial resistance to several antibiotics (Finch, 1998; Kunin, 1993). Due to increased resistance of many microorganisms towards established antibiotics, investigation of the chemical compounds with in traditional plants has become desirable (Phillipson, 1991). There are many published reports on the effectiveness of traditional herbs against Gram positive and Gram negative microorganisms and as a result, plants are still recognized as the bedrock for modern medicine to treat infectious diseases (Evans et al., 2002). Infectious disease physicians are alarmed by the prospect that effective antibiotics may not be available to treat seriously ill patients in the near future.
There is a clear evidence of revival of interest in phytomedicine at a global level, the revival which has been so dramatic that sales of herbal products in the world worth staggering over 100 billion dollars a year. China and India are two leading countries for sales in herbal products. Even in the western world popularity of the phytomedicine is increasing at a rapid pace. Germany is the leading country in Europe followed by France in the use of botanicals, around 80% of German physicians prescribe herbal medicines (Harrison, 1998).

Tannins are a large diverse group of complex polyphenolic compounds of medium to large molecular weight that are widely distributed among plants, often in bark, roots, outer layers of plant tissues, leaves, stems and seeds where they are ascribed a protective function. The principal chemical property of tannins is the ability to form strong complexes with proteins, starches and other macromolecules. Complex tannins are tannins in which a catechin unit is bound glycosidically to a gallotannin or an ellagitannin unit.

The tannins are applied widely in medicine especially in Asian (Japanese and Chinese) natural healing, the tannin containing plant extracts are used as astringents against diarrhea (Yoshida et al., 1991), antiviral, antibacterial (Haslam, 1996) and as anti-inflammatory, antiseptic and haemostatic pharmaceuticals (Haslam, 1989). Recently the tannin has attracted scientific interest, especially due to the increase incidence of deadly illnesses such as AIDS and various cancers. The search for new lead compounds for the development of novel pharmaceuticals has become increasingly important, especially as the biological action of tannin containing plant extracts (Yoshida et al., 1991).

OBJECTIVE OF THE STUDY

Currently multi drug resistant MRSA strains constitute a major health cure problem. Since they are etiological agent of several nosocomial and community acquired pathological infections. The development of resistant strains of bacteria has increased the need for new antibiotics. Bioassay guided fractionation of plant species may lead to the
discovery of new antibacterial agents and a better understanding of how ethno-medicine can treat infections.

More than 80% of the world population still depends upon traditional medicines for various skin diseases. Wound healing is a complex process characterized by homeostasis, re-epithelialisation, granulation, tissue formulation and remodeling of the extra cellular matrix. Though the healing process takes place by itself an infection can seriously delay the healing process.

The aim of this study was to identify the type of wound and S. aureus that occur in the hospital in different age group and the study also focused on several standard techniques, including recently described genotypic and phenotypic assays for the detection and identification of MDR MRSA (Multi Drug Resistance - Methicillin Resistant Staphylococcus aureus) from wound isolates and evaluated the PCR for the mecA gene identification and used different screening media supplemented with appropriate antibiotic to detect MRSA.

Most previous studies on plants for antibacterial activity were mainly performed with the extract of aerial part eg. leaves, stem, flowers, roots and rhizomes but meager with seed extracts. Hence the following seed extracts of Annona squamosa, Artocarpus heterophyllus, Cucurbita maxima, Elettaria cardamomum, Mangifera indica, Momordica charantia, Moringa oleifera, Phoenix dactylifera and Tamarindus indica were selected and screened against MDR MRSA from wound infection. Among that Mangifera indica seed ethanol extract was found to display interesting antibacterial properties against MDR MRSA, it was fractionated and the fractions were tested by checkerboard assay for Minimum Inhibition Concentration (MIC). The structural identification of the active compound was carried out by bioassay guided fractionation (chromatography), purification (High Performance Liquid Chromatography -HPLC) and spectroscopic (Infrared -IR, Mass Spectroscopy -MS and Nuclear Magnetic Resonance -NMR) methods.