As antibiotics are spine to hospital/clinical management, the emergence of strains resistant to several antibiotics of different classes in pathogenic bacteria creates utmost annoyance in microbial stewardship programme. Especially in empiric therapy, intended for acute diseases and at the end of a surgical protocol, the treating physician circumspect about the choice on antibiotics. Prescribing a moribund antibiotic would lead to the failure of the treatment, as drug resistant bacterial strains are more virulent than their sensitive strains. As it is, because of natural evolutionary capabilities of having both intrinsic and extrinsic modes of arrival of resistant strains (Perez et al., 1990), colossal emergence of MDR bacteria becomes the obvious aftermath at a blistering pace, after the introduction of some newer antibiotic along with resistance to others introduced earlier. Pathogenic bacteria evolve new strains gaining resistance to recently used antibiotics and drugs, an event which repeats by itself; and in the last few decades there have been an increase in the prevalence of MDR bacteria, worldwide. Since, bacteria have cryptic inter-continental migration, their drug resistant strains slowly escalate to other areas. Concomitant to the search for newer generations of antibiotics for the control of increased torrent of MDR pathogenic bacteria, continual efforts for search of control agents from plants, non-conventional sources and structural modifications of moribund control agents have been
undertaken (Gericke et al., 2002; Burt et al., 2004), as apothecary follows eclectic principles in
drug discovery.

MDR strains of both Gram-negative (GN) and Gram-positive (GP) bacteria have emerged increasingly as public health perils. MDR GP bacteria are less prevalent than MDR GNs, in any hospital/community setting (Falagas and Bliziotis, 2007), but species of *Staphylococcus* and *Enterococcus* spearhead as belligerent MDR GP cocci (Subedi and Bramahadathan, 2005; Sood et al., 2008), which are considered as the important determinants of public health problems, worldwide. The originally known commensal, *S. aureus* causes mild to severe or potentially fatal illness in the MRSA form, when its strains gain multiple-drug resistance; eventually, MRSA has become ill-famed as the superbug of the health domain, to put in sotto voce. As it is known, long term hospitalization causes increase of susceptibility of a patient to the MRSA infection (Chambers, 2001), particularly causing suppurations at surgical sites and invading urinary tracts. It was also reported that 51.5 % patients had already been infected with MRSA at their time of the admission to hospitals, which could cause an introduction of newer/ differently resistant MRSA strains to hospitals from community (Slonczewski and Foster, 2009).

In the surveillance of *S. aureus* this hospital, it was found that of the total 1,507 *S. aureus* isolates, 485 strains were from community and 1,022 isolates were from nosocomial sources; of 485 (100 %) community (outpatients department) *S. aureus* isolates, 390 (80.41 %) were MRSA strains. Similarly, from wards-and-cabins of 564 (100 %) isolates, 461 (81.73 %) strains were MRSA; whereas of 458 (100 %) isolates obtained from ICU and NICU, 363 (79.25 %) strains were MRSA. A progressive increase of percent values of drug resistance to 16 antibiotics of 8 groups used for antibiotic profiling revealed prevalence of MDR MRSA strains,
thereby elucidating its subtle infection dynamics in the hospital. Thus, this study revealed the appalling occurrence of MRSA as well as VRSA in a hospital of resource-limited setting.

In England and Wales, less than 2% S. aureus strains were methicillin-resistant in 1990, but in 2002 an eyebrow-raising figure, 42% strains were methicillin-resistant; approximately 300,000 cases of nosocomial MRSA infections were estimated each year leading to 5,000 deaths (Carnicer-Pont et al., 2006). Vancomycin has always been the choice of drug in the cases of MRSA infections (Nadig et al., 2006); but in this study, VISA and VRSA have been isolated, which are partially or fully resistant, respectively to vancomycin that suggested that MRSA could be resistant to vancomycin. The development of VISA is suspected due to combination therapy of vancomycin with an aminoglycoside (gentamicin) for a synergistic action of both (Raju et al., 2007).

From a careful inspection of the infection scenario of a hospital, one would be overwhelmed of accounts of pathogenic S. aureus strains imbued in wards and ICUs at least, as recorded here. Not unless one is a clinician, strive to control its pernicious infection would never come to mind with proclivity and tenacity; nor would it occur in mind unless one is a clinical microbiologist that clonal nexuses of this original commensal-avatar has become insidiously pathogenic (Mwangi et al., 2007). And the evolved strains have spiraled to an unbridled notorious standard, due to the emergence of multidrug resistance in them. Surprisingly, one would hardly find a more vivid illustration of a commensal, S. aureus, transforming into a perilous pathogen with an armamentarium of multidrug resistance, in the last few decades.

MRSA strains mostly invade during surgical procedures and the insertion of urinary catheters, which accounted for 51 and 39% of MRSA infections, respectively in a study; it was further reported that the time period of having exposures to conditions favouring the spread of
MRSA was greater than 7 or 7.4 days (Filice et al., 2010) that confirmed that these strains can spread easily and quickly in a hospital setting, and a long term hospitalization could lead to increased susceptibility to MRSA, at least because, patients admitted to ICUs are multi-morbid or have certain surgical wounds (Filice et al., 2010).

In this study, during the assessment of 278 strains resistant to 17 antibiotics, the infection-dynamics of this iconic notorious pathogen S. aureus was discernible with the minimum of 28 % resistance for vancomycin and the maximum of 97 % resistance for gentamicin. Indeed, occurrence of high percentage of resistance for daptomycin at 36 % in nosocomial samples is of high clinical concern, in this study. The most striking situation was that S. aureus strains have emerged with concomitant resistance to many commonly used antibiotics of groups seen here, also as seen elsewhere (De Leo et al., 2009). Surprisingly, the imperiling value of 30 % epidemiological prevalence of vancomycin resistant S. aureus in this hospital is a matter of concern; these could be due to errors in manual method of determining antibiotic susceptibility pattern in a resource limited settings with the absence of an automated technique, the use of vancomycin in empiric therapy and overall, the absence of a stringent antibiotic policy in local hospitals, to state contemplatively. Moreover, in an European country, of 750 clinically isolated S. aureus strains, 38 % D-test positives were obtained in CA and 67 % in nosocomial-MRSA isolates; but the D-test positive figure for nosocomial-MSSA was 63.6 %; further, MRSA isolates were often found resistant to cephalosporins, cefems and other β-lactams, ampicillin-sulbactam, amoxyclav, ticarcillin-clavulanic acid, piperacillin-tazobactam and the carbapenem, imipenem (Farrell et al., 2007). According to our survey, the percentage of D-test positivity in community acquired isolates was lower than that of nosocomial strains.
Strains that were Er-r when plated with Cd-s were expected to have D-test positivity, but out of the total 191 (Er-r, Cd-s) isolates, only 140 strains were D-test positive. Thus, a cohort of 51 Er-r strains was unable to induce D-like flattening of clindamycin-inhibition zone, due to the absence of MLSB gene. From the analysis of D-test positivity with variable factors, MRSA/MSSA, sex, absence/presence of comorbidities, etc., it was evident that the distribution pattern of MLSB gene was not universal among all Er-r isolates that could be the cause of 140 D-test positives only, among 191 Er-r strains. It is imperative that some other mechanism too is involved in Er-r, at least with 51 stains herein; that could be the active efflux mechanisms to evade antibiotics of the MLSB group by an intrinsic gene (Leclercq et al., 2002). Moreover, in this study the two types of phenotypes, D and D+, basing on the size of the clear zone around the erythromycin disc less than 6 mm for former and more than 8 mm for the later as described (Steward et al., 2005), were not detected in this study.

Enterococci are primarily opportunistic pathogens. Intensive use of broad spectrum antibiotics in the hospitals has been responsible for emergence of these organisms as important nosocomial pathogens (Gold, 2001). The first report of VRE was reported in 1988 (Uttley et al., 1988). Thereafter, VRE have spread rapidly all over the world. For example, from the year 1989 to 1993 the percentage of nosocomial infections due to VRE reported to the Centers for Disease Control and prevention, USA increased from 0.3 to 7.9 per cent. Though the major problem in treatment of VRE infection arises in endocarditis, the urinary tract is the commonest site from where bacteremia can occur. There are very few reports on isolation of VRE from India (Mathur et al., 2003), though epidemiology of nosocomial VRE bacteremia has been quite extensively studied. Studies on problems posed by the VRE as pathogens in UTI are very few. Enterococci in mixed culture are very commonly isolated from urine samples. It is not always easy to assess
the clinical significance of VRE in routine cultures or to differentiate colonization from infection (Wong et al., 2000). The present study was undertaken to look for vancomycin resistance in *Enterococci* obtained in significant numbers from various nosocomial and community acquired samples, and to study the infection dynamics of this MDR pathogen.

In the surveillance period of 30 months, of 7,634 samples, a total of 1,802 isolates were detected as *E. faecalis* and strains were isolated to pure cultures. Of these 1,802 (100 %) isolates, 600 (33.29 %) were from community sources (OPD); while 1202 (66.70 %) were from hospital sources (ICU-and-NICU, as well as wards-and-cabins).

Nosocomial acquisition and its subsequent colonization of VRE is an emerging international threat to public health, and it has been emphasized in the US; colonization of VRE among non-hospitalized persons has been also reported. In contrast, in European countries, colonization is frequently reported in persons outside the health-care setting (McDonald et al., 1997). An important factor associated with VRE in the community in Europe has been the avoparcin, a glycopeptide antimicrobial drug used for years in many European nations at sub-therapeutic doses as a growth promoter in food-producing animals. In Europe, evidence suggests that food borne VRE may cause colonization in man (Aarestrup et al., 1996; Kruse and Rorvik, 1996).

But, among 265 strains, 87 strains had clindamycin resistance with the characteristic D-flattening towards the erythromycin disc. Thus, clindamycin was ineffective in controlling Cd-s strains in the presence of Er-r character, due to presence of MLSB gene with 87, among 148 ‘Er-r, Cd-s’ strains. Clearly, the rest 61 ‘Er-r, Cd-s’ strains were lacking the MLSB gene. Further, six variable factors, it was recorded that the factor related infections and prior antibiotic uses were conducive to acquire Er-r strain with MLSB gene. The considerably high level of resistance in *E.*
faecalis isolated from HA and CA clinical samples clearly indicated that options for the use of other antibiotics of the major 9 groups remain limited. Of 14 antibiotics used, linezolid 30 μg/disc was the most effective antibiotic. Thus, the infection-dynamics was discernible, indicating *E. faecalis* as the appalling pathogen, as 265 isolated strains were floridly MDR. The increasing resistance to vancomycin had surprisingly higher levels of percentage in CA samples, compared to that of HA isolates, since Er-r strains from hospital samples were having prevalence at 80% compared to that of community samples at 57%. Thus, this proves our notion that hospital stay brings extra infections than in community.

The methylase gene, *erm(A)*, formerly termed as *erm(TR)* was identified in *S. pyogenes* conferring erythromycin resistance (Seppala et al., 1998). A work from Spain recorded different species of *Enterococcus* with erythromycin resistance, with genes, *erm(A), erm(B), erm(C), erm(TR)*, *mef(A/E)* and *msr(A)*. Each group has 2 or more variants; for example, *erm(A)* has two variants *erm(A)* and *erm(TR)*. Likewise for *erm(T)*, 17 variants of genes were recognized encoded by different transposons and plasmids (Roberts et al., 1999). Indeed, MLSB resistance in Cd-s strains of *Streptococci* carrying any version of *erm* gene is inducible to express clindamycin resistance, by the Er-r character. Besides *S. pyogenes*, other *Streptococcus* sp. such as, group C and group G species (*S. agalactia* and *S. pneumonniae*) also have *erm(A)*. Further, in the other species originally isolated from fermentation units, *Peptostreptococcus magnus* also was reported to have *erm(A)* gene (Reig et al., 2001), conferring it virulence along with drug resistance, as both characters are linked (Martinez et al., 2002). Conjugative transfer of the *erm(A)* gene from *erm(A)* positive isolates of *S. pyogenes* to Er-s strains of *S. pyogenes*, *E. faecalis* and *Listeria innocua* was recorded (Giovanetti et al., 2002). The other methylase gene, *erm(AM)* now called as *erm(B)*, coding resistance to MLSB antibiotics was associated with the
constitutive, as well as the inducible MLSB phenotypes (Giovanetti et al., 2002). This bacterial pathogen was reported to be resistance to streptogramin A too (Emborg et al., 2004). Over the last 3 decades, N6 methyl transferase had been isolated from different bacterial species, that causes DNA adenine methylation; similar methylations were recorded causing the methylation of the 23s rRNA in Enterococci and Staphylococci, which have been involved in MLSB resistance with a lot of variances, named alphabetically from \( \text{erm}(A) \) up to \( \text{erm}(Y) \) (Graham et al., 2009).

Our study corroborates similar studies from Korea, Belgium and 24 European Universal hospitals including Columbian hospitals (Schmitz et al., 2000; Lim et al., 2002; De Leener et al., 2004; Reyes et al., 2007). Enterococci particularly, \( E. \text{faecalis} \) is normally found in raw meat contaminated with fecal matter in slaughter house and a high level of MDR strains occur there on and, concomitantly contaminate surface/fresh water system too, as, if the fecal matter from slaughtered animals is not disposed of properly. Intrinsic resistance of \( E. \text{faecalis} \) had been described to a group of antibiotics including the newly adopted daptomycin and vancomycin intended for GP bacteria (Arias and Murray, 2008). Moreover, the gene \( \text{erm}(A) \) was detected late in \( E. \text{faecalis} \), but was detected early in \( S. \text{pyogenes} \) (Schwaiger et al., 2008). In an Iranian study, the prevalence of strains resistant to glycopeptides, aminoglycosides and macrolides independently and with combinations in \( E. \text{faecalis} \) and \( E. \text{faecium} \) were described along with erythromycin resistance, i.e., positive for \( \text{erm}(B) \) gene, in 45 % and 41 % of isolates, respectively. Further, both species had \( \text{erm}(A) \) gene at 5 % isolates, but \( \text{erm}(C) \) gene was not detected. And clinical isolates of \( E. \text{faecalis} \) from India were reported having \( \text{erm}(A) \) and \( \text{erm}(B) \) genes (Sood et al., 2008). In a study from Canada, it had been shown that 387 \( E. \text{faecalis} \) strains had a variety of combination of resistant genes on plasmid that caused resistance to a group of 18 antibiotics including bacitracin, chloramphenicol, flavomycin, quinupristin-dalfopristin and

Antibacterial properties of some medicinal plants against pathogenic bacteria

165
tylosin, mainly (Tremblay et al., 2011). From China 117 \textit{E. faecalis} isolates from swine yielded erythromycin resistance strains with 7 virulence genes, with resistance to tylosin and ciprofloxacin, in a large number (Zou et al., 2011). As seen, 12 \% strains were VRE consisting of 6 \% VRE \textit{E. faecalis} and 22 \% \textit{E. faecium}. Those were of the \textit{vanA} phenotype (Emaneini et al., 2008). Thus, erythromycin induced clindamycin resistant and MDR strains of \textit{E. faecalis} are present in each continent, along with increasing levels of glycopeptide resistance particularly.

In the US, \textit{Staphylococcus pyogenes} (Group A \textit{Streptococcus}) had records of nosocomial transmission, from patients to health care workers in causing fulminant invasive diseases, sore throat with hip and joint pains (Lacy and Horn, 2009). Levofloxacin resistant \textit{S. pyogenes} with MIC of 16 \(\mu\text{g/mL}\) were prevalent in the US (Richter, 2003). The multidrug resistance of \textit{S. pyogenes} was linked to major virulence factor, the M-protein, which is coded by the \textit{emm} gene (Wahl et al., 2007). It had been seen that the most GP bacteria such as, \textit{Enterococcus} sp. (including VRE), \textit{S. aureus} (including MRSA) and \textit{S. pyogenes} survive for months on dry surfaces. Many GN species such as, \textit{A. baumannii}, \textit{Citrobacter} sp., \textit{P. aeruginosa} and \textit{Proteus} sp. also survived equally for months on dry surfaces. Bacteria such as, \textit{Bordetella pertussis}, \textit{Haemophilus influenzae}, \textit{P. mirabilis} and \textit{V. cholerae} persisted only for a few days on dry surfaces. These reports clearly suggested potency of nosocomial spreads of the above mentioned bacteria (Kramer et al., 2006).

The \(\beta\)-lactam group of antibiotics comprising penicillin and its derivatives including broad spectrum cephalosporins and monobactam are hydrolyzed by ESBL producing bacterial strains. Sometimes precipitated health episodes in hospitals and communities due to GN bacteria, \textit{Citrobacter baumannii}, \textit{Proteus} sp., \textit{A. baumannii} are the uropathogens, while \textit{S. aureus} and \textit{P. aeruginosa} cause suppurations (Mittal et al., 2009). Slowly, strains of, \textit{P. aeruginosa} and
Citrobacter sp. are becoming prominent to cause invasive infections in under-5 children, alike in both developed and developing worlds because of multidrug resistance. The third generation cephalosporins (3GCs) namely, cefotaxime, ceftazidime and ceftriaxone were developed because of the production of ampicillin-hydrolyzing β-lactamases carried by plasmids, TEM1 (temoneira), TEM2 and SHV1 (sulphydryl variable). Moreover, about 150 ESBL bacterial strains had been described to have a worldwide distribution, a decade ago (Bradford, 2001) that clearly demonstrated that β-lactam antibiotic resistance emerged in geographic zones, where a particular antibiotic was used first (Jarlier et al., 1988; Reuland et al., 2013). Further, ESBL producing bacteria often show cross resistance to other groups of antibiotics like, fluoroquinolones. Further, the matter of utter clinical annoyance incidentally was the close relationship between ciprofloxacin resistance and ESBL production in antimicrobial stewardship programme (Paterson et al., 2000). Indeed, surveillance studies on a pathogen or a group provide a mirror of infection dynamics of a hospital/community. It has been known that ESBL harbouring patients require longer hospital stay in wards/ICUs, longer use of devices ventilation, catheterization and a few more that are inductive often for longer hospitalization — all stemming from as well as leading to severity of an illness arising from an infection episode and exposure to more and more nosocomial infections, followed by the use of higher generation of antibiotics (WHO, 2011; Mangeney, 2000), sometimes impassably compelling a patient’s transfer to hospice.

Spread of ESBL strains is multifactorial: they are from improperly washed hands of indurate health care providers and inanimate objects of hospitals and nursing homes, as well as the over-crowed hospital corridors and communal living settings, to cite a few. Eventually patients, who carry antibiotic resistant organisms when in contact with immunocompromised/
aged patients as well as healthcare workers cause the spread, the later often serve as reservoirs or exchangers. In indoor hospital units, viz., wards, cabins and ICUs, one infected patient is sufficient to cause infections to many, by devices, fomites and health care workers.

This surveillance has arisen out of necessity in a hospital set up, as ESBL producers create a vacuum in empiric therapy. The latter is used for several life threatening diseases and morbidities, burns and UTI cases as it takes 3-4 days for a culture reports for the real guidance for treatment since ESBL producers have evolved so much so that, the fourth generation cephalosporins (4GCs) are needed to control them. This study should guide the clinician in the final treatment as well as empiric therapy in saving patients of all age groups.

It is seen in the surveillance of GN ESBL producing bacteria that, wards and cabins of the hospital contributed more numbers of pathogenic strain compared to ICUs and NICU. Further, nosocomial total isolates in each quarter was significantly higher than total OPD isolates. Further, urine and pus samples contributed significantly higher numbers of ESBL positive strains. Antimicrobial stewardship programme usually uses certain antibiotics both in empiric therapy and regular therapy for the control of an ailment, an UTI episode for example. When an alternate antibiotic is thought of to control unbridled, notorious MDR strains of *A. baumannii* and *C. freundii* occurring in UTI patients causing cystitis and urosepsis, tigecycline was considered as an effective antibiotic for MDR and ESBL strains of these pathogens. But the difficulty in the use of an alternate higher generation antibiotic in place of tigecycline causes raised levels of side effects in host (Swoboda et al., 2008). Another problem detected with *A. baumannii* pathogenesis is its virulence due to capsular material forms of thick bundles of fibrillous structure that covers the bacterial cell surface impeding penetration of drugs in to bacterial cells (Gupta et al., 2003). In addition, adherence of 4 to 5 somatic O-antigens to host
cells by fimbrial/non-fimbrial means serves as a virulence factors; thus resistance of the bacterium to 3GC had been explained (Nathisuwan et al., 2001). In the USA even, 100 % of clinical samples of C. freundii and K. pneumoniae were susceptible to ertapenem (a carbapenem) in 2007 (Mody et al., 2007). Thus, carbapenemase originated first in a developed country.

In Japan, the occurrence of ESBL producing bacteria has been quit less compared to other countries, but in a surveillance of 7 years period from 2003 to 2009, E. coli, K. pneumoniae and P. vulgaris were quite predominance in Hakakata-ku. Japanese strains mostly comprised TEM, SHV and CTX-M plasmids (Chong et al., 2011). Nevertheless, being resistant to almost all generations of cephalosporins, Japanese E. coli strains were susceptible to carbapenems. Further, strains did not have co-resistance to aminoglycosides, but were resistant to fluoroquinolones (Chong et al., 2011).

The most striking situation is that all these bacterial strains have emerged with resistance to many commonly used antibiotic groups, aminoglycosides, macrolides, fluoroquinolones and others, chloramphenicol and tetracycline (CLSI, 2011). Further, the GNs A. baumannii and P. aeruginosa too equally acquired clonal nexuses and mobile genetic elements of multi resistance to come up to notorious standards. Surprisingly, widespread uses of antibiotics have lead to such a situation of hospital and public health pandemonium. Thus, MDR strains of A. baumannii and P. aeruginosa are the silently violent bacterial incarnations widespread in community and hospital environments and those have posed serious clinical imbroglio.

Indeed, the indiscriminate use of antibiotics could be regarded as the cause of emergence of MDR bacteria, as in regions where the availability of antibiotics is limited, the prevalence of MDR A. baumannii and P. aeruginosa were low (Tiwari et al., 2009). Thus, the problem of saturnine emergence of the cohort of MDR pathogens did not happen obliviously, but those are
welcomed by indurate attitudes in the antibiotic use, despite availability of information on mechanism of bacterial mutations and their rates.

Further, nosocomial infections are reported from ICUs, because of the severity of infection by one or other pathogen in patients on wounds. This situation causes spreads of several infectious bacteria at a time by cross-infections. Frequently, device associated nosocomial infections have been reported from many hospitals due to human errors, despite better cleanliness of general hospital environments (Eiff et al., 2005), at least in poverty stricken developing countries. Indeed, nosocomial/community spreads of infections could be attributed to the lack of general awareness among public, an indurate attitude or the lack of specific awareness among paramedical staff, and at least to the plethora of physiological and genetic survival mechanisms of MDR bacterial strains.

This thesis recorded prevalence of GP and GN bacteria to identify the present status of the philanthropic hospital attended by patients from rural areas, urban slums as well as from well heeled mass, in resource limited settings. This Indian epitome should strengthen the epidemiological database and would help fixing by facilitation of quality improvement in hospital management and for the reduction in cost of hospitalization, as well as in the reduction of morbidity and mortality due to this GP and GN MDR pathogens. The pharmacy world too is anticipated to be benefitted by this and similar studies on subtle MDR pathogens all over, for finesse in dovetailing suitable drugs of non-microbial origin even, as antimicrobials (Mesquita et al., 2007).

Thus, complementary medicines are thought up in principles of ‘comparative effectiveness research’, and isolated phyto-compounds could be promoted. Nowadays, there is an increasing interest to correlate the phytochemical compounds with their biological activities (Melendez
and Capriles, 2006). At all the time, systematic screening of plants is necessary for designing alternative/supplementary/complementary/synergistic drugs in an integrative approach, for the rapidly emerging MDR strains of pathogenic bacteria. Considerations on economics of phyto-drugs clearly indicate a promising market, a priori, waiting ahead during the fight with adept against the avalanche of MDR avatars of pathogenic bacteria and a considerable number of viral and fungal pathogens. Some large pharmaceutical company could further initiate the development of suitable supplementary/complementary drugs from phytochemicals, with sanguinity in the crusade against MDR pathogens. This thesis has unique, in comparison to most other works in the field of monitoring antimicrobial activities of medicinal plants with human pathogens, as MDR bacteria directly isolated from patients were used.

Of the two polar solvents ethanol and water, the former was found to be invariably a better solvent for almost all of 70 plants used, as ethanol plant extracts were studded with more plant metabolites than in aqueous extracts. Screening with this large number of plants could lead to a conclusion that non-edible plant species were suitable for antimicrobial activities against pathogenic bacteria. Furthermore, most of the bacteria studied herein are potential enough to cause havoc in the management of patients, because of MDR characters, to extents that would warrant alternative drug therapy. For example, *P. aeruginosa* is resistant to ampicillin, amikacin and co-trimoxazole. It could be due to the wide spread genetic recombination methods operative in nature, which may be one of many causes of multiple resistance in all pathogenic bacteria. Nosocomial infections of pathogenic bacteria including MDR *S. aureus* widespread in a hospital referred herein could be facilitating for the general exchange of genetic materials, i.e., resistance factors (Ngueyem et al., 2009).
A report from Asia reported the antibacterial activity of multi-solvent extracts of *Oroxylum indicum* bark against various Gram negative and GP bacteria. Particularly hexane, chloroform and carbon tetrachloride extracts showed significant activity against *Bacillus megaterium*, *S. paratyphi*, *Vibrio mimicus*, *V. parahaemolyticus*, *P. aeruginosa*, *B. cereus*, *B. subtilis* and *E. coli*. Another plant, *Celastrus paniculata* had antibacterial activity against *Streptococcus pyogenes*, *B. subtilis*, *B. cereus*, *Corynebacterium diphtheriae*, *S. typhi*, *S. paratyphi* A and B, *E. coli*, *P. aeruginosa*, *S. aureus*, *K. pneumoniae* and *P. vulgaris*. Furthermore, the aqueous extract of *C. paniculata* seed had potential antibacterial activity against *B. cereus*, *K. pneumoniae*, *P. vulgaris*, *S. typhi*, *S. paratyphi* A, *E. coli*, *P. aeruginosa* and *S. aureus* (Russo et al., 2001).

In a study from Mysore, India, *Woodfordia fruticosa* was reported to have antibacterial activity against the standard MTCC strains of the GP pathogens *S. aureus* and *S. faecalis* having zone of inhibition more than 21 mm, which was more than the zone of inhibition of the positive control, the antibiotic gentamicin. In the same study, the same plant showed a great deal of antibacterial activity against other standard MTCC GN bacteria, particularly against *S. paratyphi* B, *Shigella boydii* and *S. dysenteriae*. In this study, both standard MTCC strains and clinical isolates from several sources with different resistant patterns of the three GP strains were used. It was discernable that *W. fruticosa* could control the used GP bacteria *in vitro*, corroborating another study (Kumaraswamy et al., 2008).

An Indian report describes work done on *in vitro* control of MDR and ATCC strains of *E. coli*, *K. pneumoniae*, *S. mutans*, *S. bovis*, *E. faecalis*, *P. aeruginosa*, *S. aureus*, *S. typhimurium* using ethanolic extracts of five plants, *Acacia nilotica*, *Syzygium aromaticum* and *Cinnamomum zeylanicum*, *T. arjuna* and *Eucalyptus globules*. The most potent antimicrobial plant was *A. nilotica* (with a MIC range of 9.75-31.3 μg/mL) (Katkar et al., 2009).
A crude methanol extract of *Garcinia nigrolineata* had antibacterial activity against MRSA. An ethanol extract of *Garcinia kola* was tested against MRSA; MIC value of the extract was 0.08-1.8 mg/mL, while the MBC value ranged from 0.135 to 4.2 mg/mL. Thus, *G. kola* was recorded to be strongly active against MRSA (Khan et al., 2009).

A 50 % ethanol extract of the dried fruits (with chebulagic acid, chebulinic acid, corilagin, gallic acid, punicalagin, terchebulin, and terminalic acid) of *Terminalia chebula* (local Haritaki) inhibited the growth of MRSA, with a MIC value of 31.3 mg/mL. This plant, native to India has been used in traditional medicines to treat respiratory tract infections (Khan et al., 2009).

The tea tree (*Melaleuca alternifolia*) oil was reported to control 66 clinical isolates of *S. aureus*; of the isolates tested, 34 were MRSA and 32 were mupirocin-resistant *S. aureus*; the MIC and the MBC values were 0.25 % and 0.50 % diluted oil, respectively. Moreover, some of the naturally occurring compounds in the oil, including 1, 8-cineol (4.5-16.5 %), terpinen-4-01 (29-45 %), y-terpinene (10-28 %) and a-terpineol (2.7-13.0 %), were recorded to show a reduced growth pattern of MDR *S. aureus*, *in vitro*, without any bacterial resistance to the oil even at a concentration of 2.5 % (v/v) (Khan et al., 2009).

Crude extracts of 10 Brazilian pants were screened for antibacterial activity of 7 clinical MDR microorganisms utilizing as control ATCC strains. Ethanol extracts of plants, *Geissospermum argenteum*, *Uncaria guianensis*, *Brosimum acutifolium*, *Copaifera reticulate*, *Licania macrophylla*, *Ptycotelum olacoides* and *Dalbergia subcymosa* were effective against MDR *S. aureus* and MDR *P. aeruginosa* (the urinary tract pathogen), and the *S. aureus* ATCC strain 6538 (Mativandlela et al., 2008).
In a study from Chennai, India, it was reported that, among common weeds, L. camara, Antigonon leptopus, and Croton sapsiflorus had remarkable antibacterial activities against B. subtilis, K. pneumoniae, S. aureus and E. coli (the MTCC drug sensitive strain); the methanol extract of L. camara particularly, had a better antibacterial activity in comparison to 2 other solvents extracts (acetone and chloroform), as well as other plants used (Udayprakash et al., 2011). Lantadene, a triterpenoid was the first isolated toxic compound from L. camara. The Indian species of L camara had lantanoic and lantic acid, which were toxic too (Ghisalberti, 2000). Later, euphane triterpene lactones were also isolated from the methanolic extracts, which were potential inhibitors of human thrombin that help in blood clotting. The review gave a vivid description of chemicals of L. camara (Ghisalberti, 2000); a number of iridoid glycosides, furonaphtoquinones and flavonoids (3-methoxy-, 3, 7 dimethoxy and 3, 7, 49-trimethoxyquercetin), a flavon glycoside and camaraside and three new pentacyclic triterpenoids, camarin, lantacin and camarinin were isolated from the methanolic extracts of L. camara. Its phytocompounds were active against both S. aureus and S. typhi (Barre et al., 1997). Multi-solvent extracts of O. indicum had antibacterial activities on GN bacteria, particularly hexane, chloroform and carbon tetrachloride extracts showed significant activity on B. megaterium, Salmonella paratyphi, V. mimicus, V. parahaemolyticus, P. aeruginosa, B. cereus, B. subtilis and E. coli (Islam et al., 2010).

Leaf-extracts, using solvent, methanol, n-butanol, ethyl acetate and dichloromethane independently, had been recorded to have the best control activity over clinically isolated MDR 3 GP and 5 GN bacteria, in this study. Effective in vitro controls of MDR strains of GP and GN bacteria, MRSA, S. pyogenes, VRE, A. baumannii, C. freundii, P. mirabilis, P. vulgaris and P. aeruginosa (the later 5 being the most potential UTI pathogens) by extracts of the 5 plants, L.
camara, O. indicum, C. paniculatus, P. santalinus and W. fruticosa were recorded herein; these plants were frequently used by several ethnic societies for health care needs.

*L. camara* (Verbenaceae family) is a shrub-weed, commonly found in South America, Africa, Asia and Australia, with tropical and subtropical climates and is a dominating weed in Kenya. In Nigeria, leaf-incineration is traditionally used for repelling mosquitoes (Egunyomi et al., 2010). In Indian *Ayurveda*, the decoction of fresh root of the plant is used as a gargle for odontalgia. For all types of dysentery, cuts, wounds, swellings, infusion of powdered leaves is used by hilly tribes (Ghisalberti, 2000); leaves have antimicrobial and termiticidal activities (Verma and Verma, 2006). Anti-tubercular activity of this plant against MDR tubercle bacilli had been reported from Mexico (Jimenez-Arellanes et al., 2003). The antibacterial activity of methanolic leaf-extract of *L. camara* have been reported for non-resistant GP and (GN) bacterial strains, *B. subtilis, S. aureus, K. pneumoniae, P. aeruginosa* and *E. coli*, *in vitro*, from India (Udayprakash et al., 2011). Among phytochemicals of *L camara*, lantadines, anthraquinones and triterpenes were reported to have antifungal properties too (Kumar et al., 2006; Juliani et al., 2002). However, teratological effects of leaves in rats also have been recorded (Mello et al., 2005).

*O. indicum* (Bignoniaceae family) is flowering plant, commonly called, Midnight horror, kampong, or Indian trumpet flower. It is a tree which can reach a height of 12 meters (39 ft). The large leaf stalks wither and fall off the tree and collect near the base of the trunk, appearing to look like a pile of broken limb bones. The seeds are round with papery wings. The *O. indicum* seed is used in the traditional Indian Ayurvedic medicine. The root bark is used as astringent, bitter tonic, stomachic and anodyne. The leaf contains chrysin and baicalei tetuin, the 6-glucoside of baicalein, is reported in seeds. Other flavonoids, known for their anti-
inflammatory and anti-allergy effects are also present. Oroxindin has also been isolated from roots of *O. indicum* (Bisht et al., 2011).

*C. paniculatus* (Celastraceae family) is an Indian medicinal plant which, has been used for thousands of years in the traditional *Ayurvedic* system of medicine. It is gaining importance fast in the primary healthcare systems as well as, in herbal drug formulations. The oil obtained from seeds of the plant is reported to be highly beneficial in stimulating intellect and sharpening the memory. It also acts as a potential nerve tonic, rejuvenator and an anti-depressant. Moreover, the plant possesses a strong antioxidant as well as free radical scavenging activity. *C. paniculatus* has also been exploited for its potential role in the management of neurodegenerative diseases and other neuronal disorders such as Alzheimer’s disease. Oil being a powerful stimulant for neuromuscular system is also used for the treatment of rheumatism and paralysis. Detailed phytochemical composition, pharmacological properties as well as therapeutic applications of different parts of *C. paniculatus* were reviewed (Arora and Rai, 2012).

*P. santalinus* (Fabaceae family), commonly known as Red sanders, is endemic to India and is considered globally endangered, with illegal harvest being a key threat. The plant is renowned for its characteristic timber of exquisite colour, beauty and superlative technical qualities. The red wood yields a natural dye santalin, which is used in colouring pharmaceutical preparations and foodstuffs. In the traditional medicine, the decoction prepared from the heartwood is attributed to various medicinal properties. It has been used in inducing vomiting, treating eye diseases, ulcers and mental debilities. The heartwood of Red sanders is known to have antipyretic, anti-inflammatory, antihelmintic, tonic, hemorrhage, dysentery, aphrodisiac, and diaphoretic activities. It has also been used as a cooling agent. Ethanol extract of stem bark was reported to possess anti-hyperglycemic activity. The wood in combination with other phyto-
drugs is also prescribed for snake bites and scorpion stings. Phytochemical investigations of aqueous and ethanol extracts of stem bark revealed the presence of alkaloids, phenols, saponins, glycosides, flavonoids, triterpenoids, sterols, and tannins. The heart wood contains isoflavone glucosides and two anti-tumour lignans, viz., savinin and calocedrin. However, the species has remained unexplored for many pharmacological activities claimed (Walpola et al., 2011).

_W. fruticosa_ (family, Lythraceae). Its English name is Fire flame bush and it is locally known as Dhataki, locally. All parts of this plant possess valuable medicinal properties, viz., anti-microbial, anti-inflammatory, anti-tumor, hepato-protective and free radical scavenging activity. Decoction of leaves in combination with sugar and dried ginger is used for general fever (Das et al., 2007). Several compounds, macrocyclic hydrolysable tannins, anthraquinone glycosides, flavonoids (polyphenols) have been reported from this plant. Leaf and flower extracts and metabolites of this plant are known to possess several useful pharmacological activities.

In the present study, methanol extract of _W. fruticosa_ leaves were evaluated pharmacognosically and phytochemical analysis by GC-MS study was done. _W. fruticosa_ showed the best controlling activity against 8 selected bacteria (3 GPs and 5 GNs). The GC-MS analysis of n-butanol fraction of the _W. fruticosa_ revealed the presence of a number of secondary metabolites, which have therapeutic properties such as, antibacterial, antifungal, antiseptic, anthelmintic, anti-inflammatory, antihemolytic, anticancerous, antioxidant, antiparasitic, antidiabetic and wound-healing activities (Nitha et al., 2012). The compounds with higher percentages in peak areas namely, diethyl phthalate (26.77), phenol, 5-methyl-2-(1-methylethyl) (13.37), dimethylocta-2,6-diene-1-thiol (10.71), phenol,2-methoxy-4-(2-propenyl)- (3.42) and hexadecanoic acid (2.88) are present in the n-butanol fraction. These six compounds have previously been reported to have medicinal properties. Diethyl phthalate had been reported to
have antimicrobial, acetylcholinesterase and neurotoxic activity (Velanganni et al., 2011). The saturated fatty acid, hexadecanoic acid is known for a wide range of activities, viz., anticancerous, antimicrobial, antioxidant and antihemolytic activity (Agoramoorthy et al., 2007; Wei et al., 2011). The compound, phenol, 2-methoxy-4-(2-propenyl), had been reported to have antibacterial, antimicrobial, antiseptic, anesthetic and anticancerous properties (Khadem and Marles, 2010). The terpenes isolated in this study consists of phenol, 5-methyl-2-(1-methylethyl)-, phenol, 2-methoxy-4-(2-propenyl)-, 2, 6-octadien-1-ol, 3,7-dimethyl-(E)-, 2,6-octadienal, 3,7-dimethyl-, cyclohexanol, 2-methylene-5-(1-methylethenyl). Terpenes exhibit the antimicrobial activity, and mostly sesquiterpenes are reported as active against bacteria and fungi (Rao et al., 2010). The monocyclic phenolic compound, phenol, 5-methyl-2-(1-methylethyl) has antibacterial, antifungal, antiseptic and antihelmintic activities (Rao et al., 2010). Indeed, terpenes isolated were, phenol, 5-methyl-2-(1-methylethyl)-, phenol, 2-methoxy-4-(2-propenyl)-, 2, 6-octadien-1-ol, 3,7-dimethyl-(E)-, 2,6-octadienal, 3,7-dimethyl-, cyclohexanol, 2-methylene-5-(1-methylethenyl), which could have specific antimicrobial activity.

The LC$_{25}$ value of the crude leaf-extract was at 97.72 mg/L during toxicity studies with human lymphocytes, which was far more than the MBC value of n-butanol fraction. The crude extract of W. fruticosa was non-toxic to human lymphocyte cultured in vitro. Thus, this plant could possibly be helpful to the present concern of the control of MDR pathogens that are potent enough to precipitate episodes in public health. Phyto-compounds, alkaloids, glycosides, terpenoids, steroids, saponins and tannins were present in the leaf-extract, which were supposed to have contribution to the recorded control of MDR bacteria (Nascimento et al., 2000). This plant could be used as a part of integrative medicine for bacterial pathogens, as antimicrobials of non-microbial origin, with mainstream antimicrobials.