SUMMARY AND CONCLUSIONS

Since pre-history, plants have been serving as an unending source of natural medicines, which are the bastions of health and well-being till date in the marginalized section in developing countries, as in India. Phyto-compounds are lending themselves today even in the modern drug development process. On the other hand, pathogenic bacteria have been seen intractable due to the development of clonal nexuses that result in the emergence of resistance to applied antibiotics, progressively in causing clinical annoyance; antibiotic-resistant pathogenic bacteria are peripatetic and get spread in community and hospitals. Many a time, those are the causatives of several episodes of terminal illness; and those need be controlled with an iron-hand. This thesis was aimed to monitor antibacterial activity of some ethnomedicinal plants used by aborigines against pathogenic bacteria, isolated from clinical samples. Plants were collected from forest pockets of Kalahandi District, Odisha; and some of those would be helpful in the development of complementary/integrative agents to be used along with main stream medicines as non-microbial antimicrobials.

In this thesis, standard microbiological procedures for isolating bacteria from clinical samples, along with disc-diffusion and agar-well diffusion methods were used for checking antibiotic sensitivity patterns of bacteria as well as, monitoring antibacterial activity of plants,
respectively. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of plant extracts were determined using the micro-broth dilution technique. Surveillance of isolated 8 pathogenic bacteria was undertaken for assessing their infectious shenanigans in the hospital (IMS and Sum Hospital, Bhubaneswar — our collaborating center) and adjoining community sectors. According to principles of Indian pharmacopeia, physico-chemical parameters of selected plants were determined; standard procedures in biochemistry were followed for the qualitative estimations of phytochemicals. Synergistic/antagonistic interaction of an antibiotic with the crude plant extract of the leading plant was examined. In an attempt for host toxicity testing of the leading plant, toxicity to in vitro cultured lymphocytes of human cord blood was monitored in the study.

The thesis has recorded hospital-acquired (HA or nosocomial) and community-acquired (CA or outpatients department, OPD) accounts of antibiotic resistance of Gram-positive bacteria, strains of *Staphylococcus aureus* and *Enterococcus faecalis*, isolated from clinical samples of the hospital (IMS and Sum Hospital). And, it was concluded that the isolated bacteria were multidrug resistant (MDR), from the recorded information on antibiograms of bacterial strains. Bacteria were methicillin resistant *S. aureus* (MRSA), vancomycin resistant *E. faecalis* (VRE); and inducible clindamycin resistance patterns of *S. aureus* and *E. faecalis*, in the typical Indian hospital were recorded, a systematic study reported never before from India. Of the total of 1,507 *S. aureus* isolates, 485 strains were from community and 1,022 isolates were from nosocomial sources; of 485 (100 %) community sources OPD *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 ‘intensive care unit’ or ICU-and-NICU (neonatal intensive care unit), 363 (79.25 %) strains were MRSA. It was ascertained statistically
that MRSA strains were equally distributed in 'community' or 'wards-and-cabins' or 'ICU-and-NICU' sources, alike the rest other drug-resistant \textit{S. aureus} strains. Antibiotic sensitivity patterns of isolated strains with 16 antibiotics were ascertained. Of 390 (100 \%) MRSA strains isolated from OPD, 80 (20.51 \%) were vancomycin resistant \textit{S. aureus} (VRSA) and 173 (44.35 \%) strains were moderately sensitive to vancomycin or, vancomycin intermediate \textit{S. aureus} (VISA). Similarly from nosocomial sources, of 461 (100 \%) MRSA isolates obtained from wards-and-cabins, 110 (23.86 \%) strains were VRSA and 208 (45.11 \%) were VISA strains, whereas of 363 MRSA isolates obtained from ICU-and-NICU, 61 (16.8 \%) VRSA strains and 164 (45.17 \%) VISA strains were found. A progressive increase of percent values of drug resistance to 16 antibiotics of 8 groups used for antibiotic profiling revealed its subtle infection dynamics.

This study has also deliberated an attempt of surveillance in probing to the occurrence of D-test positive \textit{S. aureus} strains (erythromycin induced clindamycin resistance), in a resource-limited setting. Obviously, a post-hoc analysis on the cause of failure in to-do-away-with the MDR strains of this pathogen by an empiric treatment with any member of the macrolides, lincosamides and streptogramin B (MLSB) group, specifically the clindamycin would be a clinical misdemeanor. A heedful univariate analysis of the bivalence of D-test results with several hospital factors, sex, presence of comorbidities, etc., vindicates this study. Further, an antibiograms of a spectrum of 278 isolates of \textit{S. aureus} with 17 antibiotics was obtained that gave an idea on the prevalence of the insidious infection-dynamics and the associated shenanigans of this notorious superbug of health domain, for a benefit of apothecary in dovetailing suitable drugs and to decrease unwarranted increases in the growing cost of hospital care, in face of the intimidating erythromycin-induced MLSB resistance.
In the surveillance period of 30 months, of a total 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 (outpatients department or OPD); while 1202 (66.70 %) were from hospital sources (ICU-and-NICU, as well as wards-and-cabins). Of community sources of 600 (100 %) isolates, likewise, from sources of wards-and-cabins of 594 (100 %), as well as of ICU-and- NICU) sources of 608 (100 %) isolates. Of 600 (100 %) OPD *E. faecalis* isolates, 259 (43.16 %) were VRE strains. Similarly, from wards-and-cabins of 594 (100 %) isolates, 262 (44.10 %) strains were VRE; whereas of 608 (100 %) isolates obtained from ICU-and-NICU, 246 (40.46 %) strains were VRE. *E. faecalis* strains were resistant at 66 % vancomycin in nosocomial (hospital-acquired) settings and 68 % vancomycin resistance in community settings. In each progressive 10 quarters of 30 months of the study period, percentage of resistance most of 16 antibiotics of 8 groups steadily increased. For amikacin, the resistance percentage of community acquired pathogenic *E. faecalis* strains increased from 61 % in the first quarter, through 46 % in the second quarter to 69 % in the last quarter, in the study period. Similarly in nosocomial section, it increased from 66 to 72 % and then to 66 % in first three quarters and ultimately to 72 % in the last (tenth) quarter.

Inducible clindamycin resistance among *E. faecalis* has also surveyed in this thesis. Of 265 strains, 42 constitutive resistant (resistant to both antibiotics) strains were totally vancomycin resistant; and of 148 ‘Er-r, Cd-s’ strains, 87 (32.83 % of 265) showed D-test positivity and the rest 61 strains were D-test negative. D-test results was examined with 6 hospital factors as bivalents, of which the vancomycin sensitive *Enterococcus* (VSE)/ vancomycin resistant *Enterococcus* (VRE) bivalent and presence/absence of prior antibiotic use>90 days bivalent, were statistically significant. VRE strain with D-test positivity would be
picked up 0.5702 times more frequently than a strain with VSE and D-test positivity. Also, patients with prior antibiotic use > 90 days had 3.7375 times more chance of picking up D-test positive strains, than patients without prior antibiotic use. Resistance pattern of *E. faecalis* strains to individual 14 antibiotics were recorded; maximum values of resistance were against ampicillin 10 μg/disc for HA and CA isolates and least values were for linezolid 30 μg/disc. From Student’s *t*-test for HA and CA data of resistance, it was recorded that drug resistant strains were equally prevalent in both sources.

This study revealed the appalling state of occurrence of MRSA, VRSA and MLSB-resistant strains of *S. aureus* and *E. faecalis* in a hospital of a resource-limited setting. A progressive increase of percent values of drug resistance to 16 antibiotics of 8 groups revealed their subtle infection dynamics. In the surveillance of Gram-negative bacteria within the period of 15 months, clinical samples from patients of nosocomial sectors (ICUs, NICU, wards and cabins) as well as from patients attending OPD were used for isolation of pathogenic bacteria. It was found that the number of isolated bacteria were 1254 as nosocomial isolates, whereas those were 897 strains from OPD sources. The isolated bacteria were 407 in number in ICUs and NICU, whereas those were 847 in number from wards and cabins; thus, the total isolates in 15 months (5 quarters) were 2151. The maximum number of bacterial isolates were obtained from urine samples and other clinical samples yielded number of bacteria in decreasing trend, urine > pus > body fluids > sputum > skin swab > blood > tracheal aspirates > throat swabs > cerebrospinal fluid > stool. Each of 2151 isolates tested for extended spectrum beta-lactamase (ESBL) positivity, 1270 number of isolates were ESBL positive.

In the present thesis, ethno-medicinal information on 70 plants documented along with details of modalities about crude extracts as medicine for many ailments. Most of these plants
were lesser-known/ non-common and are in the use for infectious diseases, by aborigines. 70 plants were extracted with ethanol and water by cold percolation method. The antibacterial efficacy of aqueous and ethanol leaf extracts of all the 70 medicinal plants against the isolated 8 MDR pathogenic bacteria were tested. From the results, it can be concluded that ethanol extracts of the plants screened gave a better yield than aqueous extracts. Aqueous and ethanol extract of plants, Celastrus paniculatus, Lantana camara, Oroxylum indicum, Pterocarpus santalinus and Woodfordia fruticosa had excellent antibacterial activity, against all the 8 MDR bacteria. Therefore, these 5 plants were further extracted with 8 non-polar to polar solvent (petroleum ether, ethyl acetate, chloroform, n-hexane, acetone, methanol, ethanol and water) by hot extraction and cold percolation methods. Antibacterial activities of these plants solvent extract were checked against clinically isolated 8 MDR strains. Methanol, ethanol ethyl acetate and dichloromethane solvent extracts have shown excellent antibacterial activity against almost all bacteria and plant extracts were more effective against Gram-positive bacteria in comparison to Gram-negative bacteria. MIC and MBC were also evaluated against the above 8 bacteria. Effective in vitro control of MDR strains of S. aureus, E. faecalis, S. pyogenes, A. baumannii, C. freundii, P. mirabilis, P. vulgaris and P. aeruginosa, the most potential urinary tract infection causing bacteria by extracts of all major plants used herein was recorded. MDR S. aureus isolated from skin lesions was found to be resistant to vancomycin, oxacillin and was found sensitive to maximum plant extracts. The armada of chemotherapeutic drugs and dovetailed antibiotics against MDR pathogens plausibly could marvel and shudder the present resistant infection-problem, if the principle of synergism with suitable phytochemicals as complementary medicine in a revised therapeutic module is adopted with adept, as concluded from results.
Results of preliminary qualitative phytochemical study of crude powders of 70 plants indicated the presence of alkaloids, tannins, cardiac glycosides, steroids, flavonoids and saponins. Tannins were present in most plants, followed by glycosides and steroids. Thus, the handful of selected plants studied could be seen as potential sources of new/useful antimicrobials. The main goal of physicochemical study is to assess the pharmacognosical value of raw materials and to ensure that the final product is of the required standard. In the present thesis, the methanol extract of *W. fruticosa* Kurz. leaves were evaluated for its popular pharmacognosical evaluations by standard methods of Indian Pharmacopeia. And phytochemical analysis by gas chromatography–mass spectroscopy (GC-MS) study was done to identify the plant properly and to standardize the crude drug. Studies on biologically active compounds in *W. fruticosa* by GC-MS analysis clearly illustrated the presence of 13 compounds. 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. This study confirmed the presence of therapeutically potent antimicrobial compounds in n-butanol fraction of leaf-extract of *W. fruticosa* for the control of MDR pathogenic bacteria. Toxicity studies on *in vitro* cultured lymphocytes indicated absence of host toxicity of the plant, as evident from lethality values with lymphocytes and absence of DNA damage in comet assay due to the plant extract. This study with the most effective plant *W. fruticosa* may be followed further with the lead molecules for the development of a specific herbal formulation for the control of MDR pathogens.