Discussion
6. Discussion

Multidrug resistance in Gram positive cocci have increased at an alarming rate in clinical settings. Drug resistance in microorganisms has predictable and perhaps inescapable response to the use of antimicrobial agent. This can arise from the selection of resistant strains among naturally susceptible species or from the ingress of new strains of naturally resistant species. The extent of use of particular agents in a given environmental dictates, the rate at which resistance arises among microbial populations.

The rise of multidrug resistance has prompted an interest in the development of novel antimicrobial agents. It is not surprising that all pathogenic species have adopted survival mechanisms to counteract both old and new antimicrobials. The drive to produce new agents targeting novel sites that may circumvent resistance is critical to the long term control of bacterial infection. The bacterial membrane is an appealing target, given that most structural elements and resistance to membrane-targeting antibiotics would require major changes in the membrane structure, which may influence the permeability of barrier.

The proliferation of the nosocomial pathogen as Methicillin-Resistant Staphylococcus aureus (MRSA) promoted by poor infection control procedures and antibiotic selection pressure. Antimicrobial drug resistance is also an economic concern with an impact on doctors, patients, health-care administrators, pharmaceutical companies and the public. So the development of new antimicrobial drugs have been used to overcome the antibacterial resistance. However, the plant-derived medicines have been part of traditional health-care in most part of the world and the antimicrobial properties of plant derived compounds are well-documented. So there is an increasing interest in plants as sources of antimicrobial agents.
6.1. Preliminary Identification of Clinical Staphylococcal species

Rapid detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA) by standard clinical microbiological procedure is tedious and time consuming, since, it first requires the identification of isolated Staphylococcus aureus colonies within mixed flora samples before assessing their level of methicillin resistance.

From our present investigation, a total of 126 clinical samples were collected from the patient's age groups of 20 to 60 (in years) in various hospitals of Namakkal District, Tamil Nadu. Out of these, 50 strains as Methicillin-Sensitive *Staphylococcus aureus* (MSSA), 10 strains as Methicillin-Resistant *Staphylococcus aureus* (MRSA), 28 strains as Coagulase-Negative *Staphylococci* (CNS) and 38 samples were sterile. Methicillin resistant isolates have been confirmed through the two new chromogenic media as MeReSa Agar medium and HiCrome MeReSa Agar medium. Similarly, Prakash, *et al.*, (2007) have also collected a total of 60 clinical samples from the age group of 1 to 70 (in years). Out of these, 21 strains of MRSA, 8 strains of MSSA and 31 strains of CNS isolates have been identified. They identified the *Staphylococcus* species through Catalase test, Coagulase test, growth on nutrient agar, blood agar, Mannitol salt agar medium, biochemical reactions and disk diffusion method.

In the present research, 39.6% of Methicillin - Susceptible *Staphylococcus aureus* (MSSA), 7.9% of Methicillin - Resistant *Staphylococcus aureus* (MRSA), 22.2% of Coagulase-Negative *Staphylococci*, and 30.1% samples were sterile form out of 126 clinical samples. Similar reports have observed by Durmaz, *et al.*, (1997) and identified the 73.8% of *Staphylococcus aureus*, 31.3% of MRSA, and 26.2% of CNS out of 513 Staphylococcal species.

Summaiya Mulla, *et al.*, (2007) have been used pus, sputum and other clinical samples for the isolation of Staphylococcal species. They identified 20 numbers of *Staphylococcus aureus* and 11 of MRSA from pus samples and 3
of *S. aureus* and one of MRSA from sputum samples. From our present investigation, 22 of *S. aureus* and 4 of MRSA from Pus samples, and 8 of *S. aureus* and one of MRSA from sputum samples. Both findings showed the similar report in the identification of Methicillin Sensitive and Resistant *Staphylococcus aureus*.

It was reported that 50 (39.6%) strains of *Staphylococcus aureus* were isolated from 126 clinical samples as pus, wound swab and sputum samples and identified through the growth on Mannitol salt agar (MSA) and Blood agar medium. The same report could be identified by Uwaezuoke and Aririatu (2004) as 48 strains of *Staphylococcus aureus* were isolated from wound swab, nasal discharges, vaginal swab and urine samples and identified using both the medium. It gains support from Adebola Onanuga, *et al.*, (2005) and isolated a total of 54 (36%) *Staphylococcus aureus* isolates from urine samples of healthy women volunteers.

Mostafizur Rahman, *et al.*, (2005) have reported similar findings when compare with the present investigation. They have also collected the clinical samples as pus, wound swab, sputum and blood from the Dermatology department, and identified through Coagulase test, DNAse test, Catalase test, Urease test and carbohydrate fermentation test and observed the twenty-eight numbers of *Staphylococcus aureus*.

A total of 345 consecutive isolates of *Staphylococcus aureus* were collected by Lutuf Savas, *et al.*, (2005) out of 871 clinical samples from respiratory tract, blood, urine, catheters and surgical wound and reported the specimen rates about 26.1% from surgical wound, 28.4% from urine samples, 17.4% from burns, 15.1% from blood, 8.7% from catheters and 4.3% from other sources. In our present relevant investigation, a total of 50 isolates of *Staphylococcus aureus* and 10 isolates of Methicillin-Resistant *Staphylococcus aureus* (MRSA) the clinical samples as wound swab, pus, and sputum from various hospitals and reported the specimen rates about 38.09% from wound swab, 37.3% from pus samples and 24.6% from sputum samples. Both the
reports concluded that the many of hospital-acquired infections with *Staphylococcus aureus* and its related groups.

Sonal Saxena et al., (2003) have collected a total of 319 nasal swabs from healthy individuals and identified the *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* (MRSA) using Mannitol salt agar (MSA) medium and Oxacillin agar medium. In the present investigation, 48 wound swab samples were collected and identified the *S. aureus* using Mannitol Salt agar medium with yellow colour colonies and Methicillin-Resistant *Staphylococcus aureus* (MRSA) with MeReSa Agar medium and HiChrome MeReSa Agar medium with greenish blue and bluish green colonies respectively. Similar relevant observation made by Flayhart, et al., (2005) and used the CHROMagar MRSA agar medium with violet colour colonies.

John Merlino, et al., (1996) have been isolated the Methicillin - Susceptible *Staphylococcus aureus* (MSSA), Methicillin - Resistant *Staphylococcus aureus* (MRSA) and Coagulase-Negative *Staphylococci* using Lipovitellin-Salt-Mannitol Agar, blood agar medium and Coagulase test. The present study also used two new chromogenic media, Mannitol salt agar medium, slide and tube Coagulase test and blood agar medium. Both the observations focused and coinside similar report as the identification and differentiation of Staphylococcal species.

Kalsoom Farzana and Abdul Hameed (2006) have comprised the clinical samples of blood, pus and urine from out door and indoor patients from different wards of hospitals and identified as 155 numbers of *Staphylococcus aureus* with Brain heart infusion (BHI) broth, blood agar, MacConkey agar and Cystine-Lactose-Electrolyte deficient (CLED) medium. In the present study, out of 126 clinical samples, 60 numbers of *Staphylococcus aureus* were identified based upon the colony morphology in the blood agar medium and MacConkey agar medium.
In the present investigation, fifty strains of \textit{Staphylococcus aureus} and twenty eight strains of Coagulase-Negative \textit{Staphylococci} (CNS) may included as \textit{Staphylococcus epidermidis} in the percentage of 39.6\% and 22.2\% respectively. Similarly, Ako-Nai, \textit{et al.}, (2005) have also isolated 122 strains of \textit{Staphylococcus aureus} and 56 strains of Coagulase-Negative \textit{Staphylococci} (CNS) from skin, blood, eye, wound, and nose secretions.

Annie Felten, \textit{et al.}, (2002) have used three techniques for the detection of low-level Methicillin-Resistant \textit{Staphylococcus aureus} (MRSA) including as disk diffusion method with Cefoxitin and Moxalactam, the Vitek 2 System with oxascreen and MIC values, and MRSA-Screen Latex agglutination test. They have also used the polymerase chain reaction for the identification of \textit{mecA} gene. In this work also for the detection purpose two methods have used, for preliminary identification of Methicillin resistant isolates with sensitive \textit{Staphylococcus aureus} using chromogenic agar medium and PCR for the confirmatory identification of MRSA isolates.

In the present work, two different chromogenic media included as MeReSa Agar medium and \textit{HiChrome MeReSa Agar} medium and identified the presence of MRSA isolates based upon the colour formation as greenish blue—colonies—and—bluish—green—colour—colonies—kept—24—hrs—of—incubation—at—37°C. Similarly, the present work gain support from Jan Kluytmans, \textit{et al.}, (2002), based upon the colour formation as pink colour colonies from CHROMagar selective medium. It was also reported by Heiman Wertheim, \textit{et al.}, (2001) and have identified the MRSA clinical isolates using Phenyl Mannitol broth containing Ceftizoxime and Aztreonam by the colour change between red to orange yellow through 3 days incubation at 37°C. All the reports showed the preliminary identification of MRSA isolates based upon the colour change of the medium.
6.2. Molecular Identification of Methicillin-Resistant *Staphylococcus aureus* (MRSA)

The detection of antibiotic resistance genes requires a substantial level of specificity and sensitivity. The polymerase chain reaction (PCR) is probably the technique of choice to meet all these requirements. The PCR is a rapid method and results can be often obtained in less than 24 hours, including the sample treatment to extract and purify the target DNA, separation and visualization of PCR products on Agarose gel electrophoresis. For these reasons, PCR is adequately used to track resistance genes as well as to find specific gene mutations leading to resistance.

Aziz Japoni, *et al.*, (2004) have used the modified DNA extraction for rapid PCR detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA) from the clinical samples. The PCR products were analyzed in 1.5% Agarose gel and visualized the amplicon size of 533bp with 100bp DNA ladder marker. From the present investigation, the above mentioned protocol used to isolate genomic DNA from clinical samples and the amplified product size of 527bp were visualized to confirm the molecular identification of methicillin resistance in *Staphylococcus aureus*.

In recent, Ercis, *et al.*, (2008) have observed the 106 *mecA* positive and 142 *mecA* negative *Staphylococcus aureus* strains by Polymerase chain reaction (PCR). All the 248 *Staphylococcus aureus* strains identified the positive result through the growth on Mannitol salt agar (MSA) medium. In contrast the present work the sum of among the 60 strains of *Staphylococcus aureus* and 28 strains of Coagulase-Negative *Staphylococci* (CNS) could be isolated from 126 numbers of the clinical samples. All the *Staphylococcus aureus* strains confirmed through the growth on Mannitol salt agar (MSA) medium to produce yellow colour colonies by fermenting Mannitol. But Coagulase-negative *Staphylococci* did not ferment Mannitol. The present study has made an attempt for the comparison of 10 strains of MRSA from preliminary identification with PCR and observed three strains were *mecA* positive.
positive. Based upon both the confirmation, PCR is a ‘Gold Standard’ method to identify methicillin resistance in *Staphylococcus* species.

Arnfinn Sundsfjord, *et al.*, (2004) have standardized the genetic methods for detection of antimicrobial resistance especially methicillin resistance in *Staphylococcus aureus* and designed the primer sequences (5'-3') in the amplicon size of 527bp for the amplification of *meca* gene through polymerase chain reaction (PCR). Similar method has been employed in the present study for the molecular identification of Methicillin-Resistant *Staphylococcus aureus* (MRSA) from clinical samples and used to differentiate from the preliminary identification of MRSA isolates.

The present investigation shows that a total of 60 isolates of *Staphylococcus aureus* from different patients were isolated and identified the 10 isolates as MRSA. The identified strain could be further confirmed with PCR for the amplification of *meca* gene responsible for the methicillin resistance. From this molecular observation with PCR, three strains were identified as *meca* positive and remaining seven as *meca* negative. Of all the Methicillin-Sensitive *Staphylococcus aureus* (MSSA) clinical isolates showed as *meca* negative with the reference strains of MTCC-96 (Staphylococcal Sensitive Strain). Jureen, *et al.*, (2001) have supported the present findings and isolated a total of 109 clinical isolates of *Staphylococcus aureus* from different patients. They have identified the 52 as *meca* positive and 57 as *meca* negative with the reference strains of *meca* positive MRSA (CDC 2212) and *meca* negative MSSA (ATCC 25923).

Louie, *et al.*, (2000) have been reported the evaluation of three rapid methods for the detection of Methicillin resistance in *Staphylococcus aureus* as the PCR based identification of MRSA isolates by the amplification of *meca* gene with the product size about 533bp, Latex agglutination MRSA screen test and Oxacillin agar screen plate method. Among the above three methods, PCR based identification alone related with the present research as the amplicon size of 527bp and used to differentiate the MRSA with MSSA clinical
isolates. Similarly, Lan Mo and Qi-Nan Wang, (1997) have emphasized the amplicon size of 533bp and identified the 228 clinical isolates of Staphylococcus aureus for the presence or absence of mecA gene.

Seung, et al., (2006) have been detected the presence of the mecA gene in strains of MRSA and MSSA through PCR amplification. Total genomic DNA was obtained from Staphylococcus aureus by Phenol Chloroform extraction method using synthetic oligonucleotide primers. They confirmed the 12 strains of Methicillin-Resistant Staphylococcus aureus (MRSA) and compared with one sensitive and one resistant reference Staphylococcal strain as ATCC-25923 and ATCC-33591. The PCR based confirmation of Staphylococcal strains could be compared with MIC range value of Ampicillin and Methicillin. But in our present investigation, from the preliminary confirmation of 10 strains of MRSA, 3 strains to be amplified and compared with the growth of MRSA in the two new chromogenic media. Both the investigators have reported that the PCR based confirmation was the better confirmation of methicillin resistance compared to the other methods.

In the present study, the preliminary identification of Methicillin-Resistant Staphylococcus aureus (MRSA) from the various clinical samples could be further confirmed by the amplification of mecA gene through Polymerase Chain reaction (PCR). The confirmed MRSA clinical isolates through PCR could be compared with preliminary identification of MRSA using two new chromogenic media. From this observation, ten strains of MRSA could be identified through preliminary methods. But for the molecular identification, three strains only amplified and confirmed the presence of methicillin resistance in Staphylococcus aureus. Similar work has done by Kang-Ju Kim, et al., (2005) and reported the presence of MRSA isolates through the amplification of mecA gene and compared with the β-lactamase activity. All the thirteen strains of MRSA could be amplified except ATCC-25923 (Staphylococcal sensitive strain). But in the β-lactamase activity, only one strain showed the negative result along with the Staphylococcal sensitive strain.
6.3. Antibacterial Activity of Antibiotics against Staphylococcal species

The emergence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) strains with multidrug resistance has posed challenges in the treatment of infections. These strains do not possess additional virulence properties, but they are more infectious than the strains of Methicillin-Susceptible *Staphylococcus aureus* (MSSA), but their characteristic multidrug resistance restricts the options available to treat infections caused by these organisms. Therefore, it is necessary to carry out regular antimicrobial susceptibility testing on isolates to provide data based on the selection of appropriate and affordable antibiotics.

Toye, (2005) has exercised the antibacterial activity of antibiotics against the clinical isolates of *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli* and reported the clinical isolates of *S. aureus* was susceptible to Erythromycin (22mm), Kanamycin (21mm), Neomycin (21mm), Novobiocin (27mm) and Tetracycline (29mm). The present research also carried out and observed the similar type of report except the zone of inhibition. The clinical isolates of *Staphylococcus aureus* and MTCC-96 was susceptible to Erythromycin (26mm), Kanamycin (21mm), Neomycin (15mm) and Tetracycline (31mm).

In the year 2005, Subedi and Brahmadathan have isolated 117 strains of clinical *Staphylococcus aureus* and identified the 18 (15.4%) strains as methicillin resistant. Both the strains (MSSA and MRSA) were susceptible to erythromycin, ciprofloxacin and Gentamycin. But the penicillin was active against Methicillin-Susceptible *Staphylococcus aureus* (MRSA) and resistant to Methicillin-Resistant *Staphylococcus aureus* (MRSA). Similar report has made from the present investigation penicillin was active against MSSA clinical isolate and MTCC-96. Both investigations showed the antibiotic resistance in *Staphylococcus aureus*.
Michelle Thouverez, et al., (2003) have investigated the antibiotic susceptibility and resistant pattern of MRSA and identified as all the isolates susceptible to erythromycin and resistant to ofloxacin. The same report could be observed from our study as all the three MRSA isolates sensitive to erythromycin and resistant to ofloxacin.

Summaiya Mulla, et al., (2007) have been observed that the antibiotic sensitivity pattern of Methicillin-Resistant Staphylococcus aureus (MRSA). The selected antibiotics named as Amikacin, erythromycin, clindamycin and tetracycline was resistant to Coagulase positive Methicillin Resistant Staphylococcus aureus (CoMRSA) by Kirby-Bauer Disk diffusion method. From the present study, erythromycin and tetracycline showed better activity against MRSA. But Amikacin showed the intermediate against all the three MRSA clinical strains.

It was reported that the present research investigation, showed all the molecular identification of three Methicillin-Resistant Staphylococcus aureus (MRSA) strains possessed the resistant activity against the antibiotics named as Penicillin and Methicillin. Both the strains included as MSSA and MRSA clinical isolates sensitive to tetracycline. The similar results observed by Cooper, et al., (2002) and identified the eighteen strains of MRSA were resistant to both the antibiotics and sensitive to tetracycline. Both the investigation concluded the continuing resistance in Staphylococcus aureus.

Lutfu Savas, et al., (2005) have reported the anti-MRSA activity and identified the susceptibility of MRSA strains to penicillin (10µg/ml), Vancomycin (30µg/ml), Erythromycin (15µg/ml), Norfloxacin (10µg/ml), and Ofloxacin. But the present investigation could be contrary to the above report, as the MRSA clinical isolates could be susceptible to Erythromycin and Norfloxacin. But it would be resistant to penicillin, Vancomycin, and ofloxacin.
6.4. Anti-MRSA Activity of Plant Drugs

Recent Studies have revealed that medicinal plants from various parts of the world can provide a rich source of antibacterial activity. In India, many plant species have been used widely to cure infectious diseases especially against the clinical isolates of Methicillin-Resistant *Staphylococcus aureus* (MRSA). Such plants are available locally, are inexpensive, and have become increasingly popular.

The similar anti-MRSA activity of medicinal plants have been done by Voravuthikunchai and Kitpipit (2005) and reported the aqueous and ethanolic extracts of ten traditional Thai medicinal plants against the hospital isolates of Methicillin-Resistant *Staphylococcus aureus* (MRSA). They identified the ethanolic extract of *Garcinia mangostana*, *Punica granatum* and *Quercus infectoria* as more effective with MICs for MRSA isolates of 0.05 – 0.4, 0.2 – 0.4, and 0.2 – 0.4 mg/ml, respectively. And also they observed the MBCs for MRSA isolates were 0.1 – 0.4, 1.6 – 3.2 and 0.4 – 1.6 mg/ml, respectively. In the present investigation, three plant sources are used for anti-MRSA activity as the rhizomes of *Acorus calamus*, Whole shrub of *Tridax procumbens*, and the seeds of *Pisum sativum*. From the observations, the MIC value for the different extracts of three plants as 3.12 – 12.5, 1.6 – 25 and 3.12 – 25 mg/ml, respectively. The MBC value included as 3.12 – 12.5, 3.12 – 25 and 3.12 – 25 mg/ml, respectively.

Enzo and Susan (2002) have used the ethanolic extract of five Australian medicinal plants as leaves of *Eremophila alternifolia*, leaves of *Acacia kempeana*, leaves of *Amyema quandong* and *Eremophila duttonii*, and the stem base of *Lepidosperma viscidum*. The ethanol extract from the leaves of *Eremophila alternifolia* was the most active and reduced the number of viable cells of MRSA. Similarly, the present investigation elucidated that the ethanol extract of *Pisum sativum* showed one of the higher inhibitory activities.
In the present work, Chloroform extract of *Acorus calamus* and chloroform extract of *Pisum sativum* showed the presence of flavonoids and possessed the moderate MICs value range about 3.12 – 6.12 mg/ml. Similar report has made by Tanaka, *et al.*, (2002) as to screen 16 isoflavonoids isolated from *Erythrina variegata* against Methicillin-Resistant *Staphylococcus aureus* and exhibited the highest activity with MIC values of 3.13 – 6.25 μg/ml.

Farrukh Aqil, *et al.*, (2005) have observed the ethanolic extract of 10 Indian medicinal plants against the clinical isolates of β-lactamase producing Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Methicillin-Sensitive *Staphylococcus aureus* (MSSA). All the 10 medicinal plants showed the broad spectrum of antibacterial activity with in the inhibition zone size of 11mm to 27mm, against all the test bacteria. The antibacterial potency of crude extracts was determined in the terms of minimum inhibitory concentrations (MICs) by the tube dilution method. The MICs value of plant extracts ranged from 1.3 to 8.2 mg/ml. But the present work focused on the antibacterial activity of selected medicinal plant drug against the molecular confirmation of MRSA clinical isolates. All these three plants showed the broad spectrum of activity and zone of inhibition ranged as 11mm to 21mm. The MICs value for the various extracts ranged between 1.6mg/ml to 25mg/ml.

In the present study, the inhibitory effect of individual and mixed plant extracts against MRSA could be standardized and compared with the findings of Soetan, *et al.*, (2006). Both experimental MIC values have included as the range of 1.5 – 100mg/ml.

Hyeon-Hee Yu, *et al.*, (2005) have identified the anti-MRSA activity of berberine, the main antibacterial substances of *Coptidis rhizoma* and *Phellodendri cortex* and found out the Minimum inhibition concentrations (MICs) of berberine against MRSA ranged from 32 to 128μg/ml. Ninety percentage of inhibition of MRSA was obtained with 64μg/ml or less of
berberine. In our present investigation, the different concentration range of MICs value against MRSA clinical isolates was 0.37 to 100mg/ml.

Kabir, et al., (2005) have investigated the anti-methicillin resistant Staphylococcus aureus (MRSA) activity using Six Nigerian medicinal plants with water and ethanol extracts. Both water and ethanol extracts were effective on Methicillin-resistant Staphylococcus aureus (MRSA) and showed the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the ethanol extracts of these plants ranges from 18.2 to 24.0mg/ml and 30.4 to 37.0mg/ml respectively. In the present investigation, the ethanol extracts of Acorus calamus, Tridax procumbens and Pisum sativum showed the moderate MICs and MBCs values included as 3.12 to 6.25 mg/ml.

In 2006, Chandrasekaran, et al., have reported the antibacterial activity of the methanol and aqueous extracts of certain mangrove extracts against ten isolates of Methicillin-Resistant Staphylococcus aureus (MRSA). The zone of inhibition for methanol extract was 22mm and the aqueous extract with 19mm. In the present study, similar findings have observed from the zone of inhibition for the chloroform extract of Acorus calamus, Acetone extract of Tridax procumbens and Isopropanol extract of Pisum sativum as 19mm. All the strains of MRSA were susceptible to Vancomycin (30µg/Disc). But, in the present investigation, all the three isolated MRSA were completely susceptible to tetracycline (10µg/Disc).

Ken Katou, et al., (2005) have observed the combined effects of Panipenem and Aminoglycosides on Methicillin - Resistant Staphylococcus aureus (MRSA) and reported the minimum inhibitory concentrations (MICs) about the range of 3.13 – 12.5 mg/ml. Similarly the present work has used chloroform and ethanol extract of Acorus calamus and Tridax procumbens mixtures showed the MICs value about 3.12 – 12.5 mg/ml.
Voravuthikunchai, et al., (2007) have investigated the chloroform extract of selected Thai medicinal plants against the food borne pathogens like *Staphylococcus aureus*, Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Salmonella typhi*, *Shigella* sp. Minimum inhibitory concentration (MIC) values of *Alpinia galangal*, *Boesenbergia rotunda* and *Zingiber zerumbet* extracts against most clinical *Staphylococcus aureus* and MRSA isolates were 0.01, 0.19 and 0.79 mg/ml and the minimum bactericidal concentration (MBC) values were 0.19, 1.57 and >12.5 mg/ml, respectively. In the present research, the anti-MRSA activity of the various extracts of *Acorus calamus*, *Tridax procumbens* and *Pisum sativum* were performed. The MIC values of plant extracts against the clinical isolates of MRSA were 3.12, 1.6 and 3.12 mg/ml and the MBC values were 3.12 mg/ml for all the extracts. The results confirmed that both the study have observed the good inhibitory activity against the clinical isolates of MRSA.

In the present investigation, the preliminary Phytochemical analysis was performed with acetone, chloroform, Isopropanol and ethanol extracts of three selected plants for its active chemical constituents and revealed the presence or absence of Alkaloids, Proteins and Amino acids, Flavonoids, Anthraquinone glycosides, Tannin and Phenolic compounds, Saponins, carbohydrates and Phytosterol using quantitative analysis. The similar type of work done by Farrukh Aqil, *et al.*, (2005) and observed the preliminary phytochemical analysis of 10 Indian medicinal plants, and revealed the presence or absence of Alkaloids, Phenols, Flavonoids, Glycosides, and Saponins using TLC-Bioautography. Both the research work showed the presence or absence of some active chemical constituents.

Kabir *et al.*, (2005) have also screened phytochemicals by quantitative methods. They identified the active plant constituents like tannins, alkaloids, glycosides, carbohydrates, saponins, Anthraquinone glycosides, and flavonoids. From this study, Anthraquinone glycosides containing plants showed the better activity against the Methicillin - Resistant *Staphylococcus aureus* (MRSA).
Over the last few decades, there is an inadequate remarkable progress in research and development to prevent the virulence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) infections. In the present study, the following plants, the rhizomes of *Acorus calamus* (L.), Whole shrub of *Tridax procumbens* (L.) and the seeds of *Pisum sativum* (L.) plant species possessing vital composition to withstand against the activity of MRSA. So, there is a need of significance to be carried out in future to understand the actual composition which is present in this plant species to cure the infections caused by Methicillin-Resistant *Staphylococcus aureus* (MRSA).