Summary
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A total of 126 clinical samples as wound swab (48 – 38.09%), pus (47 – 37.3%), and sputum (31 – 24.6%) collected from the different age group of patients in various hospitals around Namakkal District. The collected samples were immediately cultured for the identification of Staphylococcal species. The collected clinical samples inoculated with Nutrient agar medium, Mannitol salt agar medium, Blood agar medium, MacConkey agar medium, DNAse Agar medium at 37°C for 12 - 24 hours for the identification of *Staphylococcus aureus* and to differentiate the Coagulase-Negative *Staphylococci* (CNS) using Coagulase test and some biochemical tests including as Catalase test, nitrate reduction test, carbohydrate fermentation test with Mannitol.

Then, Two new chromogenic media used to identify the Methicillin-Resistant *Staphylococcus aureus* (MRSA) named as MeReSa agar medium and HiCrome MeReSa agar medium (HIMEDIA, Mumbai) and formed the greenish blue colour colonies and bluish green colour colonies respectively. This was one of the preliminary identification of Methicillin-Resistant *Staphylococcus aureus* (MRSA) from clinical samples.

From the Preliminary identification of Staphylococcal clinical isolates, Fifty strains of Methicillin-Susceptible *Staphylococcus aureus* (MSSA) as 39.6%, Ten strains of Methicillin-Resistant *Staphylococcus aureus* (MRSA) as 7.9%, Twenty eight strains of Coagulase-Negative *Staphylococci* (CNS) as 22.2%, and thirty eight numbers of samples possessed no growth as 30.1%.

For the molecular confirmation of methicillin resistance, the genomic DNA isolated from all the ten strains of MRSA, one strain of MSSA, and one reference Staphylococcal sensitive strain as MTCC-96 and confirmed through 1% Agarose gel electrophoresis. All the isolated genomic DNA samples were amplified through thermal cycler (TECHNE, UK) using suitable forward and reverse oligonucleotide primer with product size of 527bp from Agile Life Science Technologies, Mumbai, India, for the amplification of *mecA* gene.
(responsible for Methicillin Resistance) and identified through Agarose gel electrophoresis with suitable 100bp ladder marker.

Both the MSSA and MTCC-96 Sensitive *Staphylococcus aureus* did not amplified and identified as methicillin sensitive strains. But from the 10 strains of MRSA preliminary clinical isolates, only three strains should be amplified and finally confirmed the presence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) through Polymerase chain reaction (PCR).

The antibacterial activity of antibiotics against Methicillin-Resistant *Staphylococcus aureus* (MRSA), Methicillin-Sensitive *Staphylococcus aureus* (MSSA) and MTCC-96 Staphyloccocal sensitive strain included as Amikacin (30μg/disc), Cephotaxime (30μg/disc), Chloramphenicol (30μg/disc), Ciprofloxacin (5μg/disc), Erythromycin (15μg/disc), Kanamycin (30μg/disc), Lincomycin (15μg/disc), Methicillin (5μg/disc), Nalidixic acid (30μg/disc), Neomycin (30μg/disc), Norfloxacin (10μg/disc), Ofloxacin (5μg/disc), Penicillin G (10Units/Disc), Rifampicin (5μg/disc), Streptomycin (10μg/disc), Tetracycline (10μg/disc), and Vancomycin (30μg/disc).

Both the MSSA strain and MTCC-96 Staphyloccocal sensitive strain were susceptible to Methicillin and Penicillin, but the three MRSA strains were resistant to both the antibiotics. All the Staphyloccocal strains were sensitive to tetracycline, Chloramphenicol, Norfloxacin, Erythromycin, and Ciprofloxacin.

Three plant sources were selected for the anti-MRSA activity included as the rhizomes of *Acorus calamus*, whole plant of *Tridax procumbens*, and the seeds of *Pisum sativum*. The selected plant sources were extracted with Acetone, Chloroform, Isopropanol, and Ethanol. The concentrations of each extract against MRSA clinical isolates were 50μg, 100μg, 150μg, 200μg, and 250μg, respectively. Then the prepared plant extract discs were used for anti-MRSA activity in individual and combination of two plants (*Acorus calamus*
with *Tridax procumbens*, *Tridax procumbens* with *Pisum sativum*, and *Pisum sativum* with *Acorus calamus*).

In the individual plant Anti-MRSA activity, the ethanol extracts of *Pisum sativum*, Isopropanol extracts of *Tridax procumbens* showed higher inhibitory effects than the other extracts. Then the chloroform extracts of *Acorus calamus* also showed higher inhibitory activity against MRSA. The Isopropanol extract of *Acorus calamus*, Chloroform extract of *Tridax procumbens*, and Acetone extract of *Pisum sativum* showed less inhibitory effects.

In the mixed plants Anti-MRSA activity, Isopropanol extract of *Tridax procumbens* with *Pisum sativum* showed the higher inhibitory activity than the other mixed plant extracts. Ethanol extract of *Pisum sativum* with *Acorus calamus*, and the ethanol extract of *Acorus calamus* with *Tridax procumbens* also showed the higher inhibitory activities. The Chloroform extract of *Acorus calamus* with *Tridax procumbens*, Acetone extract of *Tridax procumbens* with *Pisum sativum*, and Isopropanol extract of *Pisum sativum* with *Acorus calamus* showed the less inhibitory activities.

The Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) values of *Pisum sativum*, *Tridax procumbens* and *Acorus calamus* and also for mixed plants from various extracts includes, Acetone, Chloroform, Isopropanol and ethanol against MRSA.

Chloroform extract of *Acorus calamus* (6.25mg/ml), Isopropanol and Ethanol extracts of *Tridax procumbens* (3.12mg/ml) and Isopropanol and ethanol extracts of *Pisum sativum* (6.25mg/ml) had high MIC and MBC value.

The higher MIC value in mixture of two plants was in the ethanol extract of *Acorus calamus* with *Tridax procumbens* (6.25mg/ml), Isopropanol extract of *Tridax procumbens* with *Pisum sativum* (1.6mg/ml) and Ethanol extract of *Pisum sativum* with *Acorus calamus* (3.12mg/ml). The higher MBC value in mixture of two plants was in the ethanol extract of *Acorus calamus*.
with *Tridax procumbens* (6.25mg/ml), Isopropanol extract of *Tridax procumbens* with *Pisum sativum* (1.6mg/ml) and Isopropanol and Ethanol extracts of *Pisum sativum* with *Acorus calamus* (3.12mg/ml).

Various extracts collected from the plants were tested for identification of its active chemical constituents. The Preliminary Phytochemical screening of *Acorus calamus*, *Tridax procumbens* and *Pisum sativum* on different extracts have been identified. Phytochemical tests indicated the presence and absence of Alkaloids, Proteins and Amino acids, Flavonoids, Anthraquinone glycosides, Tannin and Phenolic Compounds, Saponins, Carbohydrates and Phytosterol in different plant extracts.

In various extracts of *Acorus calamus*, Acetone extract showed the presence of alkaloids, Anthraquinone glycosides, saponins and carbohydrates. Chloroform extract showed the presence of alkaloids, flavonoids, tannin and Phenolic compounds, and saponins. Isopropanol extract showed the presence of proteins and amino acids, Anthraquinone glycosides and carbohydrates. Ethanol extract showed the presence of alkaloids, flavonoids, saponins and Phytosterol.

In various extracts of *Tridax procumbens*, Acetone extract showed the presence of flavonoids only. Chloroform extract showed the presence of alkaloids only. Isopropanol extract showed the presence of Anthraquinone glycosides, carbohydrates and Phytosterol. And the Ethanol extract showed the presence of alkaloids, proteins and amino acids, tannin and Phenolic compounds and Phytosterol.

In various extracts of *Pisum sativum*, Acetone extract showed the presence of alkaloids, Anthraquinone glycosides, and carbohydrates. Chloroform extract showed the presence of flavonoids and Phytosterol. Isopropanol extract showed the presence of alkaloids, Anthraquinone glycosides, tannin and Phenolic compounds, saponins and carbohydrates. And the Ethanol extract showed the presence of alkaloids, proteins and amino acids.
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