

RESEARCH METHODOLOGY

A number of antibacterial mechanisms were studied using phenotypic approaches. For proteomic analysis, *Staphylococcus aureus* was selected as a representative species of Gram-positive bacteria. Clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) were included. Experimental protocols in this study were outline below (details were included in published papers):

Preparation of crude extracts

Preparation of semi-purified and purified compounds

Bacterial strains and growth conditions.

Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MIC)

Determination of antibacterial activity of the crude extracts and pure compounds

Studies on antibacterial mechanisms:

Study on cell surface hydrophobicity

Biofilm formation

Quorum sensing

Effect on certain enzymes

Effect on toxin production

Scanning and Transmission electron microscopy

Proteomic studies:

Bacterial cell protein preparation from rhodomyrtone-treated *S. aureus* cells

Protein profiling analysis of *S. aureus* and MRSA treated with the ethanolic extract of *Rhodomyrtus tomentosa* and rhodomyrtone by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Preparation of *S. aureus* mouse antisera

Western blotting analysis to identify *S. aureus* proteins

Preparation of 2D gel samples

Two dimensional gel electrophoresis

Image analysis.

In-gel digestion and peptide extraction.

Matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI

TOF/MS).

Liquid chromatography-mass spectrometry (LC-MS).

Database searching and verification of target identification

Applications:

Applications of crude extract in food systems