Abstract

Streptococcus mutans is known as a primary pathogen of dental caries. Glucan synthesis, adherence to tooth surface and biofilm formation is important physiological and virulence factors of S. mutans. The first two studies aimed to evaluate the influence of the crude and active solvent fraction of Trachyspermum ammi and Prosopis spicigera on S. mutans cariogenicity, effect on expression of genes involved in biofilm formation and caries development in rats. The crude extract and the solvent fraction exhibiting least MIC were selected for further experiments. Comparative gene expression analysis was carried out for nine selected genes known to be mostly involved in S. mutans biofilm formation. For the caries study, a total of 50 rats infected with S. mutans were treated topically. Real-time RT-PCR analyses revealed significant shifts in the expression of the genes involved in biofilm formation. The results presented provide new insights at the molecular level regarding gene expression in this bacterium when grown in the presence of plant extracts, allowing a better understanding of the mechanism influencing biofilm formation by S. mutans. All the test groups showed reduction in caries (smooth surface as well as sulcal surface caries) in rats. Furthermore, the data suggests the putative cariostatic properties of these plants. The study further indicates that the petroleum ether fraction of T. ammi and the ethanolic fraction of P. spicigera could be the fractions of choice for identifying further potentially novel anti-caries agents.

The objective of the third study was to isolate and characterize the active compound from Trachyspermum ammi seeds, exhibiting antibiofilm activity against Streptococcus mutans. Purification of the active compound from the seeds was performed by silica gel chromatography, and spectroscopic methods (FTIR, NMR and MS) were employed for its identification and structure determination. Antibiofilm and
anti-adherence activities of the active compound against S. mutans were analysed. Confocal microscopy was performed to visualize the effect of the compound on biofilm structure of S. mutans. Around 50% reduction was observed in adherence at 39 µg/ml and in biofilm at 78 µg/ml. It was found effective against adherent cells of S. mutans, reduced water-insoluble glucan synthesis and inhibited the reduction in pH. Confocal microscopy revealed scattered cells at sub-MIC concentration of the compound, resulting in distorted biofilm architecture in contrast to clustered cells seen in control. This study revealed a novel compound, a naphthalene derivative, isolated first time from T. ammi seeds with antibiofilm activity against S. mutans. Trachyspermum ammi represents an interesting source of a novel compound, (4aS, 5R, 8aS) 5, 8a-di-1-propyl-octa-hydroronaphthalen-1-(2H)-one, with a great potential to be used as a therapeutic agent against dental caries.

The emerging trends of multidrug resistance among several groups of microorganisms against different classes of antibiotics led different researchers to develop efficient drugs from plant sources to counter multidrug resistant strains. This study was made to determine the antimicrobial activities of the crude ethanolic extracts of five plants against multidrug resistant (MDR) strains of Escherichia coli, Klebsiella pneumoniae and Candida albicans. ATCC strains of Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis, Streptococcus bovis, Pseudomonas aeruginosa, Salmonella typhimurium, Escherichia coli, Klebsiella pneumoniae and Candida albicans were also tested. The MDR strains were sensitive to the antimicrobial activity of Acacia nilotica, Syzygium aromaticum and Cinnamum zeylanicum, whereas they exhibited strong resistance to the extracts of Terminalia arjuna and Eucalyptus globulus. Community-acquired infections showed higher
sensitivity than the nosocomial infections against these extracts. The most potent antimicrobial plant was *A. nilotica* (MIC range 9.75-313µg/ml), whereas other crude plant extracts studied in this report were found to exhibit higher MIC values than *A. nilotica* against community acquired as well as nosocomial infection. This study showed that *A. nilotica*, *C. zeylanicum* and *S. aromaticum* can be used against multidrug resistant microbes causing nosocomial and community acquired infections.

Different solvent extracts of *Prosopis spicigera*, *Zingiber officinale*, and *Trachyspermum ammi* (*T. ammi*) were investigated to determine their efficacy against multidrug resistant microbes. Successive extractions of these plants were performed using a Soxhlet apparatus, using solvents with increasing polarities. Preliminary phytochemical analysis was also performed. Minimum inhibitory concentration was determined by a two-fold serial dilution method followed by determination of minimum bactericidal/fungicidal concentration. Multidrug resistant (MDR) strains of *Candida albicans*, *Candida krusei*, *Candida tropicalis*, *Candida glabrata*, *Escherichia coli* and reference strains of *Streptococcus mutans* and *Streptococcus bovis* were used in the study. The ethanolic fraction of *P. spicigera* (least minimum inhibitory concentration [MIC] - 4.88 µg/ml) demonstrated a remarkable inhibition of the microorganisms while fractions obtained from those of *Zingiber officinale* (least MIC-78.125 µg/ml) exhibited little activity. The petroleum ether fraction of *T. ammi* (least MIC- 625 µg/ml) showed best activity when compared to its other fractions. Qualitative analysis of the phytoconstituents was also performed. This study suggested that *P. spicigera* could be a potential source of new antimicrobial agents.