Medicinal plant extracts and herbal preparations are complex mixtures of active and ballast substances which may contain numerous, not infrequently up to several hundreds of different constituents with unknown structures. Quality, safety and efficacy of these herbs are thus a great issue and their analysis is challenging. Natural extracts based drugs being a complex mixture of number of metabolites having complex and unknown mechanism of action. Rather it is difficult but very informative task to explore the mechanism of action of natural drugs. Post treatment analysis of the proteome of the test organism is one approach to explore the molecular mechanism of action of herbal drugs. Considering the vast potentiality of plants as sources to antibacterial and antifungal agents, a systematic investigation was undertaken to screen the local flora for their antimicrobial activity. Antimicrobial mechanisms of most active plant extract were investigated at molecular level by proteomic analysis of treated and control samples.

In the present study, ten plants species i.e. Picorrhiza kurroa, Datura metel, Acacia catechu, Cissus quadrangularis, Cassia tora, Berberis aristata, Pongamia pinnata, Emblica officinalis, Saraca asoca and Tinospora cordifolia were shortlisted on the basis of literature survey of traditional medicine systems. The test microorganisms used in the study includes six bacterial strains i.e. Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Escherichia coli, Klebsiella pneumonia and Pseudomonas aeruginosa and three species of Aspergillus namely, A. fumigatus, A. flavus and A. niger. The test organisms were selected on the basis of their association with various forms of human infections.

Crude extracts were prepared using soxhlet extraction and aqueous extraction methods. Percent yield of soxhlet based plants extract varies from 0.80 to 5.77%. Percent yields of petroleum ether and chloroform extracts of plant leaves was found to be in the range of 0.80 to 2.98%. Percent yields of acetone, methanol and water extract were found to ranging from 2.87 to 5.77%. Percent yield through aqueous extraction varies from 7.2% to 12.50%. Percent extract yield in case of S. asoca and B. aristata were recorded maximum i.e. 12.5 % & 12.02 % respectively, where as it is lowest in case of D. metel and P. pinnata i.e. 7.2 %.
Total sixty extracts of ten different plants were screened for antifungal activity using microbroth dilution assay. Amphotericin B, the positive control used in this study shows MICs in the range 0.73 to 1.95 µg/ml against fungal strains. Water extract of S. asoca showed maximum activity against A. fumigatus (0.65 mg/ml). The extracts with MIC ranging from 0.65 mg/ml to 2.5 mg/ml were further evaluated for their potential using disc diffusion assay. Potential antifungal activity of water extract of S. asoca against A. fumigatus was confirmed with the result that its maximum zone of inhibition at a preset concentration of 25 µg/disc was 8.0±0.5 mm against A. fumigatus. Antibacterial potential of extracts was also evaluated using Resazurine based microbroth dilution assay. Streptomycin is used in this study as positive control shows MICs in the range of 3.9 to 15.62 µg/mL against the bacterial strains used in the study. Methanol extract of C. quadrangularis showed promising activity whereas water extract of S. asoca showed maximum activity against P. aeruginosa (MIC 0.15 mg/mL). Less or negligible activity was observed in all the extracts of T. cordifolia. Aqueous and methanol extract of other plants showed antimicrobial activity in a range of 5.0 to 0.62 mg/mL. However second best activity was shown by aqueous extract of C. tora against S. aureus (0.530 mg/mL).

Leaf water extracts of S. asoca with highest activity against P. aeruginosa was selected as the most active extract. It was observed that the extract show nearly 50% haemolysis at the highest concentration used in the study (40 mg/ml) whereas standard antimicrobial drug (Streptomycin) shows 50% hemolysis at a concentration of 1.87 mg/ml. Extract was found to be 21.3 times less cytotoxic as compared to streptomycin hence it is considered to be safe for use.

Being highly potential among the studied plant extracts, S. asoca leaf water extract was further explored for its in-vitro antimicrobial mechanism by analyzing P. aeruginosa proteome. The cellular and secretory proteins which show significant differential expression under sub MIC extract stress were characterized and analyzed using 2-dimensional electrophoresis. The Factor Projection Plot of the secretory protein gels shows closer match to a given set of gels, showed a small pattern deviation and a high degree of reproducibility. A total number of 145 spots were recognized by the software in control & treated gels but only 46 (p<0.05) were found.
to be differentially regulated. Of the 46 significant protein spots only 16 spots showed significantly higher abundance (p<0.005). These 16 protein spots were digested with trypsin using in gel digestion technique and their mass spectra were obtained using highly accurate Q-TOFMS instrument. Sequences of peptides obtained from mass spectra were analyzed against non-reductant NCBI protein database by using Spectrum Mill software. Protein identification accuracy was assessed by using SPI score and percent amino acid coverage. On the basis of information generated for all the 16 spots using Spectral Mill three down-regulated proteins i.e. PstC (Spot ID-47), Trb D (Spot ID- 105), Phase 2 flagelin (Spot ID-121) are found to be important which interferes or dependent on bacterial type IV secretion system.

While studying the cellular protein profile; A total number of 396 spots were recognized by the software in control & treated gels but only 75 (p<0.05) were found to be differentially regulated. Of the 75 significant protein spots only 7 spots showed significantly higher abundance (p<0.005). These 7 protein spots were digested with trypsin using in gel digestion technique and their mass spectra were obtained using highly accurate Q-TOFMS instrument. Sequences of peptides obtained from mass spectra were analyzed against non-reductant NCBI protein database by using Spectrum Mill software. On the basis of information generated for all the 7 spots using Spectral Mill two down regulated proteins i.e. proteins trb E (Spot ID- 281) and secretary protein Hly D (Spot ID- 294) are found to significantly interact and regulate Type I secretion system.

*P. aeruginosa* secrete a number of proteins which help in biogenesis of organelles, such as pilli and flagella, nutrient acquisition, virulence, efflux of drugs and other toxins. Six highly conserved secretion systems are known to mediate protein export through the periplasmic space of Gram-negative bacteria. Protein trbE (cagE: conjugal transfer ATPase) of *P. aeruginosa* was reported to be involved in regulation of type IV secretion system. Interaction of trbE with trbD (conjugal transfer protein) is involved in regulation of IV secretion system. Type IV secretion system is highly specialized, system specific and are macromolecule exporters for the delivery of effector molecules. However, it is least characterized secretion system in *P. aeruginosa*, but current study shows the importance of system in survival of bacteria.
Down regulation of both trbE with trbD have detrimental effect on the secretion system. Due to down regulation of traE and traD, another important protein secreted via type IV secretion i.e. phase 2 flagellin which is required for flagillin synthesis and motility of bacteria was found to be down-regulated. Type I secretion pathway related protein ‘secretion protein HlyD family protein (accession no. 26988702)’ was found to be down-regulated, which help in secretion of hemolysin (bacterial toxin) directly to the medium, bypassing the periplasm. Hemolysin secretion is independent of SecA and SecY but specifically requires for dedicated transport proteins HlyB and HlyD. It has been reported that loss of hemolysin secretion significantly reduces the rate of survival of the bacteria. Another protein PstC (membrane protein component of ABC phosphate transporter) related to membrane-associated phosphate-specific transporter (Pst) complex was also found to be down-regulated. Overall, Pst system participates in phosphate uptake, cell growth and expression of virulence-associated traits.

The observation showed that water extract of *S. asoca* mainly inhibits the expression of essential and highly specific transport systems required for bacterial survival, growth and toxins secretion, through unknown mechanism. Further exploration of plant extract and specific molecules which down-regulate these transport related protein may explore novel targets or ways to develop antibacterial drugs and targets. The molecules are being identified currently and once their structure is elucidated a comprehensive Quantitative Structural Activity Relationship (QSAR) determination can be carried out in order to find an optimal combination of pharmacologically important substructure groups. Further structural correlation based studies lead the drug industries to develop compounds of more efficacy.