ABSTRACT

Emergence of resistance against many of the antibiotics commonly used for treatment of a variety of infectious diseases has assumed an alarming proportion. Infections with multiple drug resistant (MDR) *Staphylococci, Enterococci, Mycobacteria* and *Salmonella typhi* have become a major concern in the health care sector. The pandemic of Human Immunodeficiency Virus (HIV) infections has compounded the problem of MDR-tuberculosis and other pulmonary infections. This has necessitated the development of new drugs to effectively contain the problem.

Plants have been the major source of therapeutic agents that have been used in a variety of human ailments. A large number of plant species from Assam and other parts of the north-east India is known to have medicinal properties. Most of these species are not yet explored scientifically for assessment of their potentials as useful drugs. Wanton destruction of habitats and also collection of these medicinal plants have made some of these species rare, threatened or endangered. Essentially a two pronged strategy - one aimed at developing modern biotechnology based conservation methodology and the other for screening plant metabolites for discovering useful drugs from these plant species, can be adopted for scientific exploitation of these valuable bioresources.

The present study was conceived against this perspective with the following objectives

a) to establish tissue culture methods for efficient micropropagation of three medicinal plant species of Assam viz, *Adhatoda vasica, Asparagus*
racemosus and Centella asiatica which are ethnobotanically known to possess antituberculosis activities.

b) To develop suspension cultures for these species to generate secondary metabolites having anti-microbial and specifically anti-Mycobacterium tuberculosis activities.

c) To examine the anti-microbial and anti-Mycobacterium tuberculosis activities of the crude and partially purified fractions derived from these species.

Micropropagation protocols have been developed through determination of the optimum culture conditions for rapid micropropagation of Adhatoda vasica, Asparagus racemosus and Centella asiatica. The tissue cultured plants of each of these species has been successfully established in the field condition.

Biochemical investigations have been conducted to resolve, detect and quantify the known active principles in each of these species viz, vasicine, L-asparagine and asiaticoside. Biochemical assays were performed using various chromatographic techniques to quantitate the amounts of these active principles from micropropagated plant tissue, calli and suspension cultured cells.

Protocols for suspension culture of these species have been developed using callus derived cells. The objective of developing suspension culture for these species was to find out whether the calli derived cells retain the potential to generate the known active principles of these species. A most significant and important finding of these experiments was that, not only the suspension cultured cells synthesized these bioactive molecules but these were synthesized in relatively higher quantities in comparison to that in the
intact plants. There is thus, enough scope for designing appropriate bioreactors for large scale production of these compounds.

Assays were performed to determine whether any of these compounds present in the crude and partially purified fractions of the plants have any inhibitory effect on the growth of Bacillus subtilis, Psuedomonas sp, Mycobacterium tuberculosis and the eukaryotic microbe Saccharomyces sp. The crude extracts from root and callus derived from nodal explants of Asparagus racemosus have been found to inhibit completely the growth of Mycobacterium tuberculosis while there was no such antimicrobial activity against Psuedomonas sp, Bacillus subtilis and Saccharomyces sp. The leaf extracts from Adhatoda vasica completely inhibited the growth of Mycobacterium tuberculosis and Bacillus subtilis. The extract induced moderate inhibition of growth in Pseudomonas sp and had no such effect against Saccharomyces sp. While the extracts of Centella asiatica inhibited the growth of Pseudomonas sp and Bacillus subtilis to varying extents, there was no antimicrobial activity against either Saccharomyces sp or Mycobacterium tuberculosis.

The study has proved that the two species, Asparagus racemosus and Adhatoda vasica are potential sources of drugs against Mycobacterium tuberculosis and there is scope for further pharmacogenomic explorations in this direction.