Chapter V
CONCLUSIONS AND FUTURE WORK
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5.1 Conclusion

Comprehensive experimental strategies were developed in the context of the three major objectives of the present study. Development of micropropagation protocols for the three medicinally important plant species- *Adhatoda vasica*, *Centella asiatica* and *Asparagus racemosus* addressed the specific problems related to tissue browning and exudation of phenolic compounds from the explants, enhancement of shooting efficiency and very stubborn microbial contamination in the case of *Adhatoda vasica*. Microbial contamination and selection of explants have been the two principal issues addressed with respect to the micropropagation of *Centella asiatica*. The micropropagation of *Asparagus racemosus* has not been reported earlier and all relevant aspects involved in the development of micropropagation technology for this species have been considered.

The conclusions of the work pertaining to micropropagation of the three plant species can be summarised as follows:

1. The problem of phenolic exudates and callusing at the cut ends of the explants of *Adhatoda vasica* were overcome by culturing the explants in a modified MS media.

2. Tissue culture conditions have been worked out for regeneration of *Adhatoda vasica* and *Centella asiatica* using shoot tip as explants. Shooting efficiency had been enhanced using the same protocol. The
acute problems of microbial contamination with respect to tissue culture of *Adhatoda vasica* and *Centella asiatica* from the commonly used explants viz., foliar and nodal explants have been eliminated by proper selection of explant tissue in the form of shoot tips in both cases.

3. Tissue culture protocols have been developed for regeneration of *Asparagus racemosus* using nodes with axillary bus as explants. This is the first ever instance of micropropagation of *Asparagus racemosus* following tissue culture approaches.

Tissue cultured derived plantlets of *Adhatoda vasica*, *Centella asiatica* and *Asparagus racemosus* have been successfully transferred to and established in pots.

Suspension culture of plant cells for generation of secondary metabolites of importance is a general objective of any plant biotechnological strategy for medicinally important plants. A major objective of the present study centered around the following issues:

- Development of suspension culture protocols for the proliferation of plant cells from the investigated plant species.
- Generation and quantitative assessment of the active principles in the dedifferentiated cells under suspension culture conditions.

The conclusions from the various experiments on these aspects are

1. Callusing efficiencies of *Adhatoda vasica*, *Centella asiatica* and *Asparagus racemosus* from leaf (in the case of *Adhatoda vasica* and *Centella asiatica*) and spear explants (in the case of *Asparagus*...
racemosus) was determined after 30 days of inoculation. Growth studies of the calli in MS$_7$ media revealed highest growth rates in the case of *Centella asiatica*.

In *Asparagus racemosus* the growth of callus reached the exponential phase in the third week after incubation in MS$_{10}$ media (NAA + KIN = 1+1 mg/l). Callus growth of *Adhatoda vasica* was found to be lowest. Green and friable calli of *Centella asiatica* and *Asparagus racemosus* were obtained after 35 days of inoculation in MS$_7$ and MS$_{10}$ media respectively unlike in *Adhatoda vasica* where it was obtained after 56 days of inoculation.

2. Suspension culture protocols for culture of calli derived cells of *Centella asiatica* and *Asparagus racemosus* have been developed. Assessment of the growth of suspension cultured cells revealed that in *Centella asiatica* the average weight of the cell mass increased by 268.54 % FW, 946.78 % DW and 373.68 % PCV of the initial inoculum when the growth curve reached the exponential phase after 21 days of incubation; in *Asparagus racemosus* the average weight of the cell mass was 185.59 % DW of the initial inoculum after 21 days of incubation.

3. Chromatographic experiments revealed that the dedifferentiated calli cells retained the ability to drive the metabolic pathways leading to the synthesis of asiaticoside and L-asparagine. The experiments on generation of vasicine in suspension cultured cells could not be performed due to constraints of time as the callusing efficiency of the explants from *Adhatoda vasica* was found to be comparatively less.

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A very significant findings of the study was that the active principle, asiaticoside in *Centella asiatica* was found to be about 10 times higher in amount in suspension cultured cells than that in intact callus cells. Higher growth rates of cells under suspension culture conditions accounts for this enhancement.

Another interesting finding with respect to *Asparagus racemosus* was that even the spear derived calli cells were capable of producing the active principle, L-asparagine under suspension culture condition. This compound is generally localised in the root cells in intact plant. This is the first report on the use of suspension culture for production of asiaticoside and L-asparagine.

Scientific validation of the ethnobotanical and traditional knowledge based facts pertaining to effectiveness of various plant products against specific human ailments is a major aspect of study in medicinal plants. The third objective of the present study relates to experimentation to determine the anti-tuberculosis (anti-*Mycobacterium tuberculosis*) activities of the known active principles from *Adhatoda vasica*, *Centella asiatica* and *Asparagus racemosus* which are vasicine, asiaticoside and L-asparagine respectively. Two approaches have been adopted for such determination in the present study. Firstly, experiments were designed to find out the effect of plant extracts (partially purified) of these species on selected microorganisms from the prokaryotic and eukaryotic groups. Secondly, specific tests were performed using *Mycobacterium tuberculosis* H37Rv strains with the same purpose.
Conclusions from these studies are as follows:

1. The acetone leaf extract of *Adhatoda vasica* showed inhibitory effect on the growth of *Pseudomonas* spp at 1 mg/ml concentration.

2. The 1 N NaOH leaf and root extracts of *Asparagus racemosus* inhibited the growth of *Bacillus subtilis*.

3. A very significant finding was that the acetone leaf extract of *Adhatoda vasica* and 1 N NaOH root, callus and suspension cultured cell extracts of *Asparagus racemosus* completely inhibited the growth of *Mycobacterium tuberculosis*, H37Rv bacilli at 50 μg/ml concentration.

There is no previous report on the anti-*Mycobacterium tuberculosis* H37Rv activity of *Asparagus racemosus* derived metabolites.

5.2 Future work

The present work can be extended further to address three critical aspects.

Firstly, having proved that the dedifferentiated calli derived cells of *Asparagus racemosus* do synthesise the active principles under tissue culture conditions, strategies can be developed for scaling up of the production levels of the compound on a sustained basis using bioreactors.

Secondly, specific studies can be undertaken for identifying the key enzyme (s) regulating the metabolic pathways leading to the synthesis of vasicine and L-asparagine. This would enable development of appropriate molecular biotechnological approaches for subsequent metabolic pathway engineering for enhanced generation of the compounds. Even structure determination and purification of the active compound having anti-tubercolic activity such as vasicine and L-asparagine can be undertaken.
Thirdly, having proved the anti-\textit{Mycobacterium tuberculosis} H37Rv activities of the plant products from \textit{Adhatoda vasica} and \textit{Asparagus racemosus}, specific tests have to be designed and performed on \textit{in vivo} situations involving infected animal model systems.