Medicinal plants are potential renewable natural resources and are generally considered to play a beneficial role in human health care. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds are alkaloids, flavonoids, tannins and phenolic compounds. In India, Tamil Nadu is under strategic geographical location and possesses an invaluable treasure of medicinal plants holding a major share in cultivation and export.

*B. monnieri* (Brahmi) is one of the important medicinal plant belonging to the family Scrophulariaceae. Micropropagation techniques offer new avenues for the improvement of this important medicinal plant.

The thesis entitled “Micropropagation of *B. monnieri* (L.) Pennell and application of some efficient microbes” deals with a morphometric characteristics, *in vitro* culture regeneration, organogenesis and qualitative phytochemical analysis in *in vitro* planlets such as alkaloid, flavonoid, saponins, tannins, phytosterols, phenols and carbohydrates and HPLC analysis of callus and *in vitro* plantlets of *B. monnieri* and their antibacterial activity against four human pathogenic bacteria.

Efforts were made to standardize an efficient protocol for micropropagation of this valuable medicinal plant with enhanced *in vitro* regeneration. Both direct and callus mediated regeneration were achieved from all the explants tested. Among the different explants
investigated for direct regeneration, maximum percentage of regeneration and number of shoots per explant were obtained from the nodal explant on MS medium supplemented with BAP (1.0 mg/l) and NAA (0.5 mg/l). It was followed by leaf explants.

Among the different explants cultured for callus mediated regeneration, nodal explant produced maximum amount of callus on MS medium containing BAP and 2, 4-D (each 1.0 mg/l). These calli on the MS medium containing BAP (1.0 mg/l) and NAA (0.5 mg/l) produced maximum number of shoots during the process of subculturing. Maximum shoot elongation was recorded from the shoots of nodal explants (8.4 cm) on MS medium containing GA₃ (1.5 mg/l), BAP (1.0 mg/l) and KN (1.0 mg/l).

The regenerated shoots from all the explants were responded well for rooting on MS medium supplemented with IBA or IAA (1.0 mg/l).

During the process of hardening, the rooted plantlets transferred to the sterilized mixture of garden soil, FYM and sand (ratio 2:1:1) showed 90% survival. Combined application of *Azotobacter chroococcum*, *Pseudomonas striata* and *Glomus aggregatum* showed better growth and biomass production of the regenerated plantlets.

GC-MS analysis revealed the presence of 06 compounds in the methanolic extract of *in vitro* plantlets sample, as per the report of earlier literature, these phytochemical compounds are known to have various medicinal properties.
HPLC analysis of callus shows that the three important flavonoids were identified as Quercetin, Rutin and Gallic acid. HPLC analysis of *in vitro* plantlets of *B. monnieri* shows maximum response and analysed their phytochemical constituents *viz.*, bacoside A₃, bacopaside II, bacopasaponin C isomer, bacopasaponin C and bacopaside I. All the above phytocompounds are biologically very important and active principles of this medicinal plant.

Among 9 extraction methods studies, the highest yield of total saponins (19.28±0.12%) was obtained from Method 9. In this procedure, Brahmi was soaked with water for 24 hrs. Thereafter the water was squeezed out and the pre-wetted plant material was percolated with ethanol. The method has proven to be practical for commercial proposes.

Methanolic extract of *B. monnieri* (*in vitro* leaves) showed maximum zone of inhibition against multidrug resistant strains of 4 common human pathogenic bacteria.

Thus the findings of the present investigation may be potentially used in the mass propagation of this medicinally important herbal plant through *in vitro* regeneration and subsequent improved organic cultivation technology, and also to offer protection against disease causing microorganisms in human beings.
6. 1. CONCLUSION

Contrary to earlier reports of the use and need of very high concentrations of cytokinins for Brahmi growth, the present work has deciphered methods of improving in vitro propagation by developing a novel improved protocol highlighting efficient reproducible and reliable techniques for mass multiplication of a medicinally and economically important herb *B. monnieri*.

The use of only cytokinins without any other growth hormones in low concentration in shoot induction and MS solid media used in rooting forms the highly significant observations of the study and found to be great importance in maintaining efficiency in multiplication, shortened time span, simplicity and benefit of genetic stability. This protocol is novel because of its minimal requirements and cost effectiveness for propagation. Also it is the first report of the plant being maintained in MS media with different hormones. This plant is a creeper so the possibilities of it being maintained it tanks in MS culture media forms a very attractive concept instead of growing them in pots.