Summary

The plant kingdom provides a wide variety of bioactive compounds with diverse chemical structures and massive array of biological activities. As medicinal plants receive increased scientific and commercial attention, the increasing human and livestock populations have affected the status of wild plants and there is increasing pressure on the wild plant population. Most medicinal plants harvested have placed heavily and many medicinal plants at risk of extinction. Therefore, there is a need to develope approaches to ensure the availability of raw material of consistent quality from regular and viable sources. The potent medicinally important phytochemicals are produced by combining the traditional medicinal knowledge with new research tools available. *Celastrus paniculatus* Willd. belongs to the family *Celastraceae* extremely important medicinal plant having remarkable reputation in pharmaceutical point of view. The plant roots revealed an important triterpenoid named Celastrol, which plays an important role in neurological disorders. On the other hand, availability of raw materials of these species is limited. In this connection, application of Plant Tissue Culture and other biotechnological strategies has immense potential in the large-scale propagation and conservation of the unexplored biodiversity of plants all over the world and especially large country like India. This is especially true with plants having important medicinal properties. The present study deals with i*n vitro* propagation of *C. paniculatus*, synthesis of nanoparticles by using seed coat exudates, establishment of adventitious and hairy root cultures, studies on growth kinetics for root culture, influence of AgNPs on enhancement Celastrol compound through root culture and efficacy of Celastrol on SH-SY5Y neuroblastoma cells. The significance of the work are given below.

- Half-strength Murashige and Skoog (MS) media supplemented with GA_3 showed the maximum percentage (82.4 ± 0.50) of embryo response through the embryo rescue method to increase the seed germination.

- In, direct regeneration, the highest response (87 percent) with 3.8 shoot number was achieved in 1.0 mg L^{-1} BAP, and shoot multiplication was achieved with BAP (1.0 mg L^{-1}) alongwith meta-Topolin (1.0 mg L^{-1}) at highest response (91.8 percent) of response with 10.2 shoots.

- MS medium supplemented with 0.5 mg L^{-1} BAP + 0.5 mg L^{-1} NAA was found suitable for induction of friable callus with percentage response of 91.66±0.69 and 0.5 mg L^{-1} BAP + 0.3 mg L^{-1} 2, 4-D was found suitable for hard compact callus of 89.33±1.11 response.

- In, Indirect regeneration highest shoot multiplication was achieved at 1.0 mgl^{-1} of BAP treated with meta-topolin (1.0 mg L^{-1}) in additives with 83.33 response and 7.12 number shoots.

- Among various concentrations (0.1 to 1.0 mg $L^{-1)}$ of auxins (IAA, IBA and NAA) tested for rooting, the half-strength MS medium supplemented with IBA at 0.3 mg L^{-1} showed 91 percent rooting response in direct regeneration. Whereas, in In-direct regeneration 89.66 rooting response was attained.

- Antioxidant potential was determined in BAP + NAA and BAP + 2,4-D derived calli and BAP with NAA derived calli exhibits highest phenolics (36.11±1.15 nmol of GAE/g DW), flavonoids (20.16±0.25) and maximum polyphenol accumulation.

- The IC_{50} value of DPPH scavenging ability of BAP+NAA was 66 µg/ml whereas that for BAP+2,4-D was 75 µg/ml. A lower IC_{50} value indicates maximum DPPH activity. Whereas for ABTS assays are almost similar, the IC_{50} values of the ABTS radical scavenging activity for BA+NAA being 56 µg/ml and that for BA+2,4-D being 66 µg/ml.

- The reducing power of the antioxidants increases with increasing concentration. The sequence for this reducing power is BHT ≥ BAP + NAA ≥ BAP + 2,4-D.

- This study reveals BAP + NAA had the most potent and prominent for NO scavenging activity with the g value 2.0048 by Electron Spin Resonance Spectroscopy.

- Simple, green protocol was developed for synthesis of nanosilver by using seed coat exudates. The size and shape of the nanoparticles were identified by UV–Vis spectroscopy, Powder X-ray Diffraction (PXRD), Transmission Electron Microscopy (TEM), and selected-area electron diffraction (SAED).

- FTIR, SERS conformed that silver nitrate particles are capped with bioactive molecules from exudates and may act as precursors in silver nitrate reduction from the metallic state (Ag^{+}) to the atomic state (Ag^{0}).

- Nanosilver exhibits excellent bactericidal and biofilm inhibition for selected gram-positive and gram-negative bacteria.

- MS medium supplemented with 0.3 mg l^{-1} IAA exhibits maximum (69 %) of response was found suitable for adventitious root induction. Whereas at 0.4 mg L^{-1} IBA exhibits (59.33 %) of root response.

- Hairy roots were successfully induced with 10.86 percentage of efficiency by co cultivation of leaf explants with *Agrobacterium rhizogenes* strain MTCC 532.

- Transformation efficiency was increased from 10.86 to 15.28 by supplemented with acetyosyringone (100 µM) to the half strength medium and Molecular confirmations of the transformed hairy roots were confirmed by PCR analysis with 423 bp *rol B* gene.

- Synthesized nanoparticles in the present study were used as elicitor and elicitor was added to the culture medium on 28^{th} day in the concertation ranging from (5, 10, 15 and 20 µg/ml) to the adventitious and hairy roots and incubated at various time (0,24, 48 and 72) intervals. The maximum level of fresh weight in adventitious roots FW (4.89 g), DW (0.15), whereas in case of hairy roots fresh weight (6.02 g) and dry weight (0.21 g) was recorded

- Among the concentrations (5, 10, 15 and 20 µg/ml) added to the medium, Nanosilver treated at 10 µg/ml concentration produced the highest rate of biomass content. Among the different time intervals tested, 10 µg/ml of AgNPs with 48 h exposure time resulted in enhanced biomass accumulation in adventitious roots FW (13.55 g), DW (0.64 g), hairy roots FW (15.73 g), DW (0.75g) was observed.

- HPLC analysis confirmed that, Celastrol content 2.24 folds higher (13.544 mg g^{-1} DW) in hairy root under the influence of AgNPs in comparison with untreated (6.034 mg^{-1}DW)

- While in adventitious roots, in comparison with control (4.646 mg g^{-1} DW), 1.87 folds increase was observed in silver nanoparticles treated cultures

(8.71 mg g^{-1} DW). overall results indicating that, there is a significant increase i.e. 1.55 folds of Celastrol content was observed in hairy root in comparison with adventitious root treated with AgNPs.

- SEM analysis concluded that nanosilver plays a significant role in enhancing the compound as the particles appear on both external and internal surfaces of the root.

- The radicle trapping activity 43.24, 20.51, 31.51 and 14.05 percent in hairy roots treated, untreated and adventitious roots treated and untreated with nanosilver was observed respectively.

- Cell viability assay results indicated that Celastrol compound did not showed negligible or no cytotoxic effects. The concentration from 40 μM till 200 μM had 80% of cell viability. Whereas, rotenone exhibited a dose dependent toxicity.

- SH-SY5Y cells exposure to rotenone (50 nM for 48 h) caused a significant increase in intracellular ROS levels. Interestingly, cells pre-treated with Celastrol (40 μM) markedly reduce rotenone induce ROS production.

- The SHSY5Y cells on treatment with Celastrol compound showed intact membrane compared to control thus conferring protection.