SUMMARY AND CONCLUSION

Plants synthesize and protect an assortment of biochemical items, numerous of which can be extracted and utilized as chemical feed stocks or as crude material for several scientific examinations. Many secondary metabolites produced by plants are commercially important and find use in a number of pharmaceutical applications. Numerous medications are acquired either from the entire plant or from various organs like leaves, stem, bark, root, flower and seed. Many current research are focused on identification of molecules from natural products primarily on plants since they can be sourced more effortlessly and be designated in light of their ethno-therapeutic uses.

In the present investigation, an efficient and reproducible regeneration protocol via indirect organogenesis of *Hybanthus enneaspermus* were reported. The method is flexible, which allows incorporation of different concentration of 2,4-D, NAA, BAP, Kn and IBA effective in both callus induction, multiple shoot buds proliferation and rooting. MS medium supplemented with 2.0mg/l 2,4-D + 0.8mg/l BAP was the best for callus induction, 2.5mg/l BAP + 0.6mg/l NAA was best for highest frequency of shoot proliferation and half strength MS medium fortified with 1.5mg/l IBA was found to be the best treatment for root formation. These *in vitro* studies afford an effectual technique for preservation and proliferation of this over exploited valuable medicinal plant species. Biotechnological methods are requisite in future which could also promote its medicinal use.

In the present study, the phytochemical constituents of *in vivo* and *in vitro* *Hybanthus enneaspermus* were extracted using different solvents namely petroleum ether, chloroform, acetone and carbinol. The yield of the extract of petroleum ether, chloroform, acetone and carbinol was 15, 21, 12.5 and 17 % respectively. Preliminary

phytochemical screening results of different extracts of *H. enneaspermus* was performed and it showed the presence of alkaloids, flavoniods, saponin, fixed oil, carbohydrates, phytosterol and coumarins.

The GC-MS analysis of the *in vitro* leaves of carbinolic extract of *H. ennespermus* exposed the existence of 15 different phytochemicals. Of these 15 compounds, Dodecanoic acid, methyl ester (27.223), Methyl tetradecanoate (12.407), 3-Trifluoroacetoxypentadecane (11.938), 2-Methyl-Z,Z-3,13-octadecadienol (11.471), 2-Cyclohexylpiperidine(7.862) and n-Hexadecanoic acid (7.566) are major compounds present in the *in vitro* leaf extract of *H. ennespermus.*

Biosynthesis of nanoparticles was a vital territory in the field of nanotechnology which has financial and environmentally friendly advantages over physical and chemical methods of synthesis. In the present work, synthesis of ZnO nanoparticles was obtained by using 1 mM Zinc (II) nitrate and CeO_2 nanoparticles was obtained by using 1 mM Cerium (III) nitrate were reduced to metallic zinc oxide and cerium oxide nanoparticles respectively on reaction with aqueous *in vitro* leaf extract of *Hybanthus enneaspermus* resulting in synthesis of ZnO NPs and CeO2 NPs. The biosynthesized zinc oxide nanoparticles (ZnONP) and cerium oxide nanoparticles (CeO_2NP) were characterized by UV visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), X- ray Photon Spectroscopy (XPS), Scanning Electron Microscopy (SEM) - Energy dispersive X-ray analysis (EDX), High resonance -Transmission electron microscopy (HR-TEM) and Selected Area (Electron) Diffraction (SAED) pattern.

The cytotoxic effect of the *in vitro* leaf extract of *H. enneaspermus* capped with ZnO NPs and CeO2 NPs was examined on human breast cancer cells (MCF-7) by

exposing cells for 24 hand 48 h. *In vitro* cytotoxic activity of green synthesized ZnO NPs and CeO2 NPs against human breast cancer (MCF-7) cell line was remarkable with 50% of mortality at 10µg/ml. The results of *in vitro* cytotoxicity assay stated that ZnO NPs and CeO2 NPs synthesized from *in vitro* leaf of *H. enneaspermus* exhibited increased growth inhibition on MCF-7 cancer cells. Further, addition of ZnO NPs and CeO2 NPs at concentrations of 10 µg/ml caused the apoptosis of MCF-7 cancer cells which is supported by the fluorescent micrographs portraying the occurrence of late apoptotic MCF-7 cells. Fluorescent staining can practicably be applied to assess apoptosis in malignant tumors. AO/EB is a more economical and convenient method to detect apoptotic cell at early/late stage.

Acetone and carbinol *in vitro* leaf extracts of *H. enneaspermus* were screened for antimicrobial activity against *Escherichia coli* (MTCC 443), *Salmonella typhi* (MTCC 98), *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 1035), *Proteus vulgaris* (MTCC 1771), *Staphylococcus aureus* (MTCC 29213), *Streptococcus faecalis* (MTCC 0459), *Enterococcus faecalis* (MTCC 2729), yeast, *Candida albicans* (MTCC 183) and fungus *Cryptococcus neoformans* (MTCC 1346) by disc diffusion assay. Among the extracts analyzed for antimicrobial activity, carbinol extract possessed good inhibition against all microorganisms tested. Gram-negative bacteria are more vulnerable to extracts of *H. enneaspermus* than Gram-positive bacteria.

Thus, the results presented in this study prove that ZnO NPs and CeO2 NPs synthesized from *in vitro* propagated leaf of *H. enneaspermus* act as effective inducers of apoptosis of cancer cells. This is an important strategy to create new anticancer therapeutics with promising potentials in clinical practice.