5. Summary and Conclusion

The present investigation focused on evaluation of the immunomodulatory potential and immunomodulation mediated anticancer activity of compounds from *Brugmansia suaveolens* and *Nicandra physalodes* (Family: Solanaceae). These plants were evaluated for their ability to stimulate both arms of the immune response, by characterizing various cellular and molecular markers in different immunologically relevant tissues such as splenocytes, macrophages and blood of mice treated with each of the test materials. Plants were also evaluated for immunomodulation mediated anticancer activity using a panel of in vitro experiments.

The results presented here in show that *Brugmansia suaveolens* and *Nicandra physalodes* were extracted and fractionated into different alkaloidal (BS1 and NP1) and non alkaloidal (BS2, BS3, BS4 and NP2, NP3 & NP4). All the fractions were subjected to bioactivity guided fractionation using *in vitro* immunomodulatory assay on human PBMC. Four most active fractions BS 3A, BS3B, NP3A and NP3B isolated by column chromatography were subjected for a panel of in vivo immunomodulatory studies using Balb/C mice at 50 and 100mg/Kg. The results revealed that all the four fractions (BS 3A, BS3B, NP3A and NP3B) augment the immune responses to T-dependent antigen (SRBC). Our findings strongly suggested that BS 3A, BS3B, NP3A and NP3B induced increase in the levels of serum anti-SRBC immunoglobulins, as measured by hemagglutination and the maximum effect was observed at 100mg/Kg. Besides, treatment with BS 3A, BS3B, NP3A and NP3B enhanced the delay type hypersensitivity reaction, as reflected by the increased footpad thickness compared to the control group, suggesting heightened infiltration of macrophages to the inflammatory site. the above data suggest that BS 3A, BS3B, NP3A and NP3B enhance both humoral as well as cell mediated immunity.

All the four fractions (BS 3A, BS3B, NP3A and NP3B) were evaluated for enhancing phagocytic activity of reticuloendothelial cells using carbon clearance assay. Enhanced response to carbon clearance was evident by a significantly high rate of phagocytic function of reticuloendothelial cells compared with control group.

Further, BS 3A, BS3B, NP3A and NP3B were screened for macrophase function using NO production assay. Increased response to NO production signify the stimulation of immune function of macrophages via NO which could be due to IFN-γ and macrophage
specific IL-12 release, which played a dominant role in the augmentation of macrophage function. By releasing cytokines and other mediators, activated macrophages influence the activity of other immune cells of the body. Also BS3A, BS3B, NP3A and NP3B increased the levels of intracellular IFN-γ, IL-2 and IL-4 in whole blood. The enhanced production of cytokines such as IL-2 and IFN-γ, which have been considered crucial for inducing a Th1 and CD8+ T cell responses and enhancement of IL-4 level regulates the Th2 immune response.

Moreover, BS3A, BS3B, NP3A and NP3B were also screened for lysosomal enzyme activity. The enhanced lysosomal enzyme activity suggest that enzyme may participate in microbial destruction. Also the enhanced response to B-cells as well as T-cells was evident by a significantly high rate of lymphocyte proliferation compared with control group.

The active fractions BS3B and NP3B were then subjected for semi-preparative HPLC to isolate the pure compounds from them and two pure compounds (SUPH036-022A and SUPH036 022B) were isolated. After isolation all compounds were subjected for various spectroscopic techniques like IR, Mass, HPLC and NMR to characterize the isolated compounds. The structure of the isolated compounds were elucidated using the above mentioned spectroscopic techniques. Both compounds were subjected for a panel of in vitro studies to screen them for immunomodulation mediated anti-cancer activity. SUPH036-022A and SUPH036 022B (2.5µg/mL) were subjected for stimulation of effector cells (human PBMC) and effector cells were cultured with target breast cancer cells (MCF-7) at various T:E ratio (1:2.5, 1:5, 1:10, 1:20). The stimulated effector cells kills the target cancer cells significantly and the maximum inhibition was observed at 1:10 (T:E) ratio.

SUPH036-022A and SUPH036 022B were evaluated for generation of reactive oxygen species (ROS) as it play an important role in decreasing ΔΨ_m and apoptosis induction. Target cancer cells were co-cultured with stimulated effector cells for 6 hours before staining with rhodamine 123. Enhanced level of ROS indicates the shift in mitochondrial membrane potential across the membrane which further leads to the initiation of apoptosis. SUPH036-022A and SUPH036 022B treated effector cells were also co-cultured with target cells for 6 hours and then target cells were screened for the loss of mitochondrial membrane potential. Decrease in the mitochondrial potential
indicates the opening of mitochondrial PTP which in turn, conduct the cytochrome c release.

Moreover, SUPH036-022A and SUPH036 022B were evaluated for cytokine induction in treated human PBMC for 48 hours. Increased in the level of IFN-γ and IL-2 in supernatant of stimulated PBMC indicates that it intensifies the weakened immune response of the body to strengthen it. Cytokines directly stimulate immune effector and stromal cells at the tumor site and enhance the recognition of tumor cell by cytotoxic effector cells.

Further, the target cells were cultured with effector cells for 24 hours and then screened for cell cycle analysis. The results indicated that SUPH036-022A and SUPH036 022B stimulated effector cells at 1:10 ratio exhibited induction of apoptosis by increase in sub-G1 DNA fraction.

In conclusion, it is clear from this study that all four fractions BS 3A, BS3B, NP3A and NP3B PBC enhanced both Th1 and Th2 immune responses to T-dependent antigen (SRBC) in BALB/c mice as revealed by the enhanced IgG/IgM titres. DTH reaction, Con-A and LPS induced T- and B-cell proliferation, enhanced phagocytic activity, NO production, carbon clearance, increased release of IFN-γ, IL-2 and IL-4 in mice sera treated with different doses of BS 3A, BS3B, NP3A and NP3B with the optimum response at 100 mg/Kg, thereby playing an important role in the modulation of the immune response. However, detailed studies is required which might establish a possible use in immunocompromised individuals.

SUPH036-022A and SUPH036 022B exhibits promising immunomodulation mediated anticancer activity against breast cancer by treating stimulated effector cells with target breast cancer cells. It inhibits breast cancer cell growth in microcytotoxicity assay, also responsible for the production of cytokines which strengthen the immune system against the tumor growth. It also increase the production of intracellular peroxides (ROS) which further disrupt mitochondrial electron transport by decreasing the mitochondrial membrane potential which further leads to induction of apoptosis. It also exhibit inhibition cell cycle in SUB/G1 phase in breast cancer cells. These results provide the basis for further in-depth drug targeted studies, while the apoptotic feature of SUPH036-022A and SUPH036 022B stimulated immune system raises the potential use of SUPH036-022A and SUPH036 022B as an antitumor agent.