Chapter 6: Summary and conclusion

6.1 Summary

The lyophilized Vipera russelli and Bungarus caeruleus venom was procured from Irula Snake Catcher’s Society, Kancheepuram, Chennai. The plants for the study A. tetracantha and C. spinarum were collected from Chitradurga and Shimoga, Karnataka respectively. The material was authenticated at National institute of Ayurveda and Dietitics, Bangalore.

The leaves of both the plants were washed, shade dried, extracted serially using petroleum ether, hexane, chloroform, ethyl acetate, methanol and water (non polar to polar) solvents in soxhlet extractor and the residue was obtained from vacuum evaporation. The extracts of A. tetracantha and C. spinarum inhibited the 5' nucleotidase, acetylcholinesterase, phosphodiesterase, phosphomonoesterase, phospholipase A\textsubscript{2} and hyaluronidase enzyme activities of V. russelli and B. caeruleus venom at 100 $\mu$g/mL. Among all the extracts ethyl acetate extract of A. tetracantha and methanol extract of the C. spinarum were found to be active extracts.

The active extracts of the plants were studied for inhibition of the toxic enzymes in dose dependent manner. It was found that all the enzymes were inhibited except L-Amino acid oxidase. The protease of B. caeruleus was not well inhibited by both the extracts.

The active extracts did not inhibit the procoagulant activity of V. russelli effectively; however the fibrinogenolytic activity of both venoms was completely neutralized by the extracts. The extract did not have profound effect on the direct hemolytic activity of the venoms and the indirect hemolytic activity was reduced to minimum by both the extracts.

The plant extracts were found to be non toxic in both mice and chick embryo model. The LD\textsubscript{50} value of B. caeruleus and V. russelli venom was 200 $\mu$g/kg and 266.7 $\mu$g/kg body weight of mice respectively. The lethal toxicity in mice was inhibited by the active extracts in pre-incubation and independent injection protocols.

The LD\textsubscript{50} value of B. caeruleus and V. russelli was 2.6 $\mu$g/egg and 3 $\mu$g/egg in chick embryo respectively. The active extracts from both the plants neutralized the lethal activity of the venom.

The V. russelli induced hemorrhage in both mice and egg; whereas B. caeruleus did not. The edema and myotoxic activity induced by the both venom were reduced by the active extracts of plants.

The fractions purified from the active extracts were not so effective in neutralizing the activities of the enzymes; hence synergistic effect of the phytochemicals to neutralize the activity of venom has been justified.
The hyaluronidase enzyme was purified from the *B. caeruleus* by reverse HPLC. The molecular weight of the enzyme was found to be 14±2 as deduced from SDS PAGE, Zymography and confirmed by mass spectrometry. The enzyme was active in pH 6, at 37°C, inhibited by metal ions and group specific reagents. The active extracts of the plants were able to inhibit the activity of enzyme.
6.2 Conclusion

Snake bite has been considered as an occupational hazard throughout the world. Mortality due to snake bite has been prevalent even in this advanced century. ASV being the only treatment available has not been able to reduce the deaths due to snake bite. It is because of non availability, side effects and logistics associated with ASV. Hence the scientific community has been looking for an alternative to reduce the death rate and also side effects. In the present study attempt has been made to provide a scientific validation to the claims of ethnobotanists and traditional healers belief of plants being the antidote for snake bite.

The studies confirm that the plants/extracts of *A. tetracantha* and *C. spinarum* possess antiophidian properties. It also adds to the already existing knowledge of synergistic effect of plant components. The study provides scientific validation to the antisnake venom properties of *A. tetracantha* and *C. spinarum* by traditional healers/ ethanobotanists. The study proves that the herbal antidotes could be used as an alternative to the antivenom at the time of need. The alternative model (chick embryo) used helps in determination of lethality and prevents excessive suffering of animal models (mice) and reduces the usage of experimental animals.