5 Summary and Conclusion

The potential of marine derived compounds is as vast as the expanse of the oceans. Marine invertebrates especially sea stars are potential yet unexplored sources of numerous bioactive agents. Of the many sea stars that the Arabian Sea harbors, the *Astropecten indicus* species is most commonly found along the west coast of India. Despite its abundant occurrence along the coast, the *Astropecten indicus* remains largely unexplored.

This study focussed on the use of the coelomic fluid (SCF) of *Astropecten indicus* as a source of novel thrombolytic drugs and wound healing agents. Thrombosis is a cardio-vascular disorder and a leading cause of mortality world-wide. Present day thrombolytic and anticoagulant drugs are associated with side effects such as uncontrolled bleeding leading to hemorrhage or non-specific mode of action. There is thus a demand for novel anticoagulants with controlled activity that target specific coagulation factors.

It was observed that crude SCF contained many proteins and polypeptides, which affected the human cardiovascular system. The crude coelomic fluid (SCF), promoted platelet aggregation as observed using whole blood platelet aggregometer and scanning electron microscopic analysis. It lysed fibrinogen and fibrin in a dose and time dependent manner. The SCF thus affected the human blood coagulation system by affecting the platelets, fibrinogen and fibrin. The major proteases that imparted these activities to SCF were metallo-proteinase in nature. The SCF did not alter the morphology of A549, HEK293 and HaCaT cultured cell lines *in vitro* and rather increased cellular proliferation upon treatment with SCF. Scratch wounds drawn on the surface of cultured cells showed faster wound closure rates post treatment with SCF compared to untreated cells. The thrombolytic and wound healing potential of SCF was thus discovered.

A novel protein AiP1 was purified from SCF by a combination of anion exchange chromatography and size exclusion HPLC. This is the first protein isolated from the sea-star *Astropecten indicus* and was found to sustain the fibrinogenolytic and fibrinolytic activities. It inhibited ADP and collagen induced platelet aggregation. The proteolytic activity of AiP1 sustained till a temperature of 70˚C and was inhibited at temperatures above 70˚C and in presence of EDTA. AiP1

promoted cell proliferation and accelerated wound healing in cancerous as well as normal cell lines cultured *in vitro*. Additionally, AiP1 was devoid of hemolytic, phospholipase and laminin digestion activities. This renders AiP1 a possibly safe candidate to be explored in drug design and cosmetic industries. Characterisation of trypsin digested AiP1 using bio-informatics tools revealed homology of one of its peptides (peptide1) to the vWFA type domain. This protein family is important for various biological processes such as platelet aggregation, blood coagulation, DNA repair, cell adhesion and cell migration. Some vWFA domains are known to contain a metal ion dependent binding site and a binding site for plasma protease ADAMTS13 (a disintegrin and a metalloproteinase with thrombospondin type 1 motif, member 13).

A combination of experimental and computational analysis of AiP1 confirms its potential as a thrombolytic and wound healing agent. This is the first report on the potential of coelomic fluid of *Astropecten indicus* as a thrombolytic and wound healing agent and AiP1 is the first protein isolated from this species and charaterised for various bioactivities.

Some important contributions emerging from this study are as follows:

1. A novel spectrophotometric method for the real estimation of fibrinolytic activity of a compound was developed which is simple, fast and economic.
2. An unexplored marine organism, the *Astropecten indicus* was reported for its thrombolytic and wound healing potential
3. A novel protein, AiP1, was isolated from SCF which aided in thrombolysis and wound healing.
4. AiP1 contained a vWF-A like domain which could be responsible for its bioactivities.