

Acknowledgements

First and foremost I thank the lord Almighty for guiding and protecting me in every walk of my personal and professional life.

I take this opportunity to express my profound gratitude and deep regards to my supervising guide and mentor Dr.Suseela Mathew, HOD, Department of Biochemistry and Nutrition Division, CIFT for her inspiring and excellent guidance, valuable suggestions, ceaseless encouragement and intellectual support. She has been my constant inspiration throughout the investigation and I am deeply obliged to her for providing all the necessary facilities and above all the absolute freedom provided in doing the experiments. I record my deep sense of gratitude to her for all the efforts she has put in and the moral support extended for the successful completion of this thesis.

I express my deep gratitude to Dr. George Ninan, Senior Scientist, Fish Processing Division, CIFT for his valuable advices, kind support, motivation and critical comments during this study.

I thankfully acknowledge Dr.Sreenivasa Gopal, Red. Director, CIFT for the entire prospect, encouragement, support and the facilities provided in CIFT for the smooth conduct of my research.

I wish to express my gratitude to Dr. Ravisankar, Director, and CIFT for the prospectus and encouragement.

I gratefully acknowledge the Department of Biotechnology, Govt. of India, and New Delhi for awarding the financial support as junior and senior fellowship for the successful completion of the work.

I am most grateful to Scientists of Biochemistry and Nutrition Division Dr. Anandan, Dr. Asha, Dr. Niladri for their valuable advices, supports enabling the successful completion of the work. My collective and individual acknowledgement is also for the technical supports, Dr. Usha Rani, Ms. Ramani, Mr. Mathai, Ms. Jaya, Ms Lekha, Mr. Suresh, Mr. Sivan at Biochemistry Nutrition Division. Special thanks to Mr. Reghu for helping me to complete the official procedures of the work.

I am immensely thankful to Dr. Ganesan, technical officer, CIFT for the valuable supports for conducting in vivo studies. It is his constant involvement even sundays helped the successful completion of the animal studies.

I take this opportunity to thank Dr. Asha Nair, Scientist, RGCB, for providing facilities and motivating me into the fascinating world of cell lines and cell culture studies. I sincerely thank the staff of RGCB for helping me to get expertise in cell culture works.

I am immensely thankful to scientists of Microbiology and biotechnology division, CIFT, for providing the necessary facilities at various stages of my work.

I wish to express my hearty thanks to Sri. Joshy George, Scientist, CIFT for providing valuable suggestions and guidance in the statistical analysis of the wealth of data generated.

I extend my sincere thanks to Librarian and staffs of libraries of CIFT and RGCB for their kind support and consideration during my literature survey.

Thanks to all administrative staff of CIFT for their great help throughout my research period.

I fondly cherish the love, care and support of my dear friends Shyni, Remya, Jones, Nabajyothy, Texin, Navaneeth, Ajeesh, Vishnu, Biji, Anju, Ananthanarayanan, Nithin, Meenu, Pradeep, Dhiju and I remember all my friends for their support and enjoyable moments we had together during this couple of years.

I would like to put on record one and all who directly and indirectly extended their kind co-operation and support throughout my research work.

I express my deep sense of gratitude and regard, to my parents and my brothers for their prayers, affection and love. My Acha and Amma have provided me with the best of all in my life, and the encouragement and support smoothly paved my path towards the successful completion of the work. I express my heartfelt gratitude to my father in law and mother in law for the encouragement, support and the patience extended towards me.

Last but not the least, thanks are due to my beloved husband Vipin Raj for his unreserved and unconditional love, prayers, moral support and motivation at all stages of my work and without his generous support this work would not have been successfully completed.

My heartfelt apologies to my son Devaprayag, for unknowingly sacrificing the beautiful moments we could otherwise have shared together.

Finally, I humbly bow before the almighty God for showering his blessings upon me and giving me the strength, wisdom, health, and luck to accomplish this important milestone in my academic life.

Hema.G.S

CONTENTS

Chapter 1

Introduction and Review of Literature

1.1	General Introduction	1
1.2	Significance of the study	3
1.3	Objectives of the study	3
1.4	Review of Literature	4
1.4.1	The Collagen Molecule	5
1.4.1.1	Distribution and molecular structure	5
1.4.1.2	Collagen types	6
1.4.1.3	Fish Collagen	9
1.4.2	Isolation and purification of collagen from fishes	10
1.4.2.1	Acid soluble collagen	10
1.4.2.2	Enzyme treated collagen	11
1.4.2.3	Insoluble collagen	12
1.4.3	Characteristics of fish collagen	13
1.4.3.1	Amino acid composition of collagen	14
1.4.3.2	Viscosity of collagen	15
1.4.3.3	Solubility of collagen	16
1.4.4	Characteristics of fish collagen to be used as biomaterial	17
1.4.4.1	Biocompatibility	18
1.4.4.2	Biodegradability	19
1.4.5	Collagen based biomaterials	20
1.4.5.1	Types of collagen based biomaterials	20

1.4.6	Wound Healing	21
1.4.6.1	Biochemical processes in wound healing	21
1.4.6.2	Moist wound healing theory	24
1.4.6.3	Collagen hydrogel	24
1.4.6.4	Collagen as wound healing biopolymer	25
1.4.6.5	Immunology and biocompatibility of xenogenic collagen material	27
1.4.7	Fish Collagen Hydrolysate	29
1.4.7.1	Collagen hydrolysate production	30
1.4.7.2	Optimisation of hydrolysate production	31
1.4.7.3	Purification and identification of bioactive peptides	32
1.4.7.4	Bioactive properties and application of collagen hydrolysate	34
1.4.8	Role in bone and joint diseases – Arthritis	36
1.4.8.1	Autoantibodies and anti CCP assay	37
1.4.8.2	Cyclooxygenases	39
1.4.8.3	Chondroprotectives	39

Chapter 2

	<i>Isolation and characterisation of collagen from different species of fishes</i>	43
2.1	Introduction	43
2.2	Materials and Methods	47
2.2.1	Raw materials	47
2.2.2	Collagen extraction	47
2.2.2.1	Pre-treatment of skin	47
2.2.2.2	Acid extraction	47
2.2.2.3	Salt precipitation and dialysis	48