

**COMPARATIVE BIOCHEMICAL COMPOSITION AND EXTRACTION OF
CHITOSAN FROM COMMERCIALY IMPORTANT CRAB SPECIES
(*Portunus pelagicus* and *Portunus sanguinolentus*)**

PROJECT WORK

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Science degree in Biochemistry

By

ABINAYA G (41070001)



**SCHOOL OF BIO AND CHEMICAL ENGINEERING
DEPARTMENT OF BIOTECHNOLOGY**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

CATEGORY – 1 UNIVERSITY BY UGC

Accredited with Grade “A++” by NAAC | 12B Status by UGC | Approved by AICTE

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI – 600 119

APRIL 2024



SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)

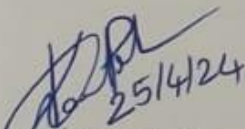
Accredited "A++" Grade by NAAC | 12B Status by UGC | Approved by AICTE

www.sathyabama.ac.in

DEPARTMENT OF BIOTECHNOLOGY

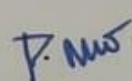
BONAFIDE CERTIFICATE

This is to certify that this project Report is the bonafide work of **ABINAYA G (41070001)** who carried out the project entitled "**COMPARATIVE BIOCHEMICAL COMPOSITION AND EXTRACTION OF CHITOSAN FROM COMMERCIALY IMPORTANT CRAB SPECIES (*Portunus pelagicus* and *Portunus sanguinolentus*)**" Under our supervision from January 2024 to April 2024.


25/4/24

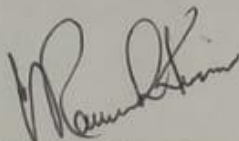
Internal Guide

MS. A. KAVIPRABA



External Guide

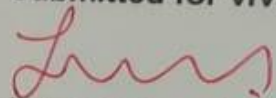
Dr. P. MARIMUTHU



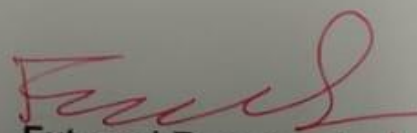
Head of the Department

Dr. V. RAMESH KUMAR

Submitted for viva - voce Examination held on


27/4/24,
Internal Examiner

(Dr. S. JAYASHREE)


27/4/2024
External Examiner

Dr. Rakash

DECLARATION

I, **Abinaya G (41070001)**, hereby declare that the project Report entitled “**COMPARATIVE BIOCHEMICAL COMPOSITION AND EXTRACTION OF CHITOSAN FROM COMMERCIALY IMPORTANT CRAB SPECIES (*Portunus pelagicus* and *Portunus sanguinolentus*)**” done by me under the guidance of **Ms. A. Kavipraba** Assistant Professor Internal Guide, Department of Biotechnology, and **Dr. P. Marimuthu** Assistant professor (Research) External Guide at centre for ocean research, is submitted in partial fulfillment of the requirements for the award of Bachelor of Science degree in Biochemistry.

DATE:

Abinaya

PLACE: CHENNAI

SIGNATURE OF THE CANDIDATE

ACKNOWLEDGEMENT

I am pleased to acknowledge our sincere thanks to The Board of Management of **SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY** for their encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my sincere thanks to **Dr. REKHA CHAKRAVARTHI, Dean, School of Science and Humanities** and **Dr. V RAMESH KUMAR, M.Phil., Ph.D., Head of the Department, Department of Biotechnology** for providing me necessary support during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my internal Project Guide **MS. A. KAVIPRABA, M. Tech** Assistant Professor, Department of Biotechnology and external project guide **Dr. P. MARIMUTHU, Ph.D.,** Assistant Professor Research, Center for Ocean Research for their valuable guidance, suggestions and constant encouragement paving way for the successful completion of my project work.

I wish to express my thanks to **all Teaching and Non-teaching staff** members of the Department of Biotechnology and Centre for Ocean Research, Sathyabama Research Park, who were helpful in many ways for the completion of the project.

ABSTRACT

The Crab meat's exquisite flavor and high nutritious content, the crab fishery in India is expanding quickly. Studies on the biochemistry of crabs are crucial to understanding their diet. Therefore, the proximate composition of protein, fat, and carbohydrates in *Portunus pelagicus* and *Portunus sanguinolentus* were assessed in the current study. The samples were collected from the Pudukkottai coastal Region. The normal techniques were followed in order to investigate the proximate makeup. The current study's findings showed that the crab of *P. pelagicus* and *P. sanguinolentus* had significant levels of moisture (80.8%), (84.6%) fat (2.19%), (1.92%), protein (18.12%) and (16.3%), and carbohydrates (0.65%) and (1.12%) respectively. The waste produced by the seafood business is massive and has a detrimental effect on the environment. Seafood trash frequently contains chitosan, which can be processed to produce goods with additional value and economic significance. The purpose of this study was to create and evaluate chitosan from crab shells for use as a food industry clarifying agent. We gathered various types of crab shells from *P. pelagicus* and *P. sanguinolentus*. After chitosan was chemically isolated, its structural and physicochemical characteristics were investigated. Evaluation was done on its application in antibacterial activity. We tested the chitosan powder's antibacterial efficacy against *Aeromonas hydrophillia*. The zone of inhibition was formed around 0.9mm of *P. pelagicus* and 0.8mm of *P. sanguinolentus*.

Keywords: *Portunus pelagicus*, *Portunus sanguinolentus*, Proximate composition, Crab shell chitosan, Antimicrobial activity

LIST OF CONTENTS

CHAPTER NO	TITLE	PAGE NO
	ABSTRACT	iv
	CONTENTS	v
	LIST OF ABBREVIATIONS	vii
	LIST OF FIGURES	viii
	LIST OF TABLES	x
1	INTRODUCTION	1
	1.1 Chitosan extraction and Antimicrobial activity	6
2	LITERATURE SURVEY	9
3	AIM AND OBJECTIVE	14
	3.1 Aim	14
	3.2 Objective	14
4	MATERIAL AND METHODS	15
	4.1 Sample collection and Dissection	15
	4.2 Proximate Analysis	15
	4.2.1 Determination of Moisture content by AOAC method	16
	4.2.2 Estimation of Carbohydrate by Anthrone method	17
	4.2.3 Determination of Protein by Lowry's method	19
	4.2.4 Determination of Fat by Bligh and Dyer's method	20

	4.3 Chitosan Extraction from Crab shell	22
	4.3.1 Deproteinization	22
	4.3.2 Demineralization	22
	4.3.3 Decolouration and Dewatering	23
	4.3.4 Deacetylation of Chitin	23
4.4	4.4 Antimicrobial activity of Chitosan	25
4.5	4.5 Charecterization of Crab Chitosan	27
	4.5.1 Fourier- Transform infrared spectroscopy Analysis	27
	4.5.2 Solubility Test	27
	4.5.3 Scanning Electron Microscopy Analysis	28
5	RESULUTS AND DISCUSSION	29
	5.1 Proximate Analysis	30
	5.2 Chitosan Extraction	31
	5.3 FTIR Analysis	33
	5.4 Solubility Analysis	35
	5.5 SEM Analysis	36
	5.6 Antimicrobial Activity	37
6	SUMMARY AND CONCLUSION	38
	REFERENCES	39

LIST OF ABBREVIATIONS

AOAC	Association of Official Agricultural Chemistry
DA	Deacetylation
DHA	Docosahexaenoic Acid
DPPH	2,2- Diphenyl – picrylhydrazyl
DTG	Derivative Thermogravimetry
EAA	Essential amino Acids
EPA	Eicosapentaenoic Acid
FESEM	Field Emission Scanning Electron Microscope
FTIR	Fourier -Transform Infrared Spectroscopy
HCL	Hydro Chloric Acid
MBC	Minimal Bacterial Concentration
NA	Nutrient Agar
NEAA	Non - Essential Amino Acids
PUFAs	Polyunsaturated Fatty Acids
SFAs	Saturated Fatty Acids
TBA	Thiobarbituric Acid
TGA	Thermal Gravimetric Analysis
TMA-N	Trimethylamine Nitrogen
USFAS	Unsaturated Fatty Acids
XRD	X -Ray Powder Diffraction

LIST OF FIGURES

Figure No	Figure Name	Page No
1.1	Chitosan structure	6
4.1.1	<i>Portunus sanguinolentus</i>	15
4.1.2	<i>Portunus pelagicus</i>	15
4.2.1	Moisture Estimation of <i>Portunus pelagicus</i> (Blue Swimmer Crab)	16
4.2.2	Moisture Estimation of <i>Portunus sanguinolentus</i> (Three Spotted Crab)	16
4.2.3	Carbohydrate Estimation (<i>Portunus sanguinolentus</i>)	18
4.2.4	Carbohydrate Estimation (<i>Portunus Pelagicus</i>)	18
4.2.5	Results were calculated using microwell plate reader for both (a) and (b)	18
4.2.6	Analytical Reagent	20
4.2.7	Follin's Reagent	20
4.2.8	Protein Estimation	20
4.2.9	Chloroform – Methanol Separation	21
4.2.10	Chloroform into Aluminium dish for total fat Estimation	21
4.3.1	Before Deprotenization	23
4.3.2	After Deprotenization	23
4.3.3	Before Demineralization	24
4.3.4	After Demineralization	24
4.3.5	Before Decolourization and Dewatering	25
4.3.6	After Decolourization and Dewatering (Chitin)	25
4.3.7	Before Deacetylation	25
4.4.1	<i>Aeromonas hydrophila</i> coated into the Nutrient Broth	26

4.4.2	Nutrient Agar	26
4.4.3	Solidified Nutrient Agar	27
4.4.4	Agar Well Diffusion Method	27
5.1.1	Biochemical value of <i>P. pelagicus</i> (Blue Swimmer Crab)	30
5.1.2	Biochemical value of <i>P. sanguinolentus</i> (Three Spotted Crab)	31
5.2.1	Chitosan yield of <i>P. pelagicus</i> and <i>P. sanguinolentus</i>	32
5.2.2	Utilization of <i>P. Pelagicus</i> and <i>P. sanguinolentus</i> to obtain Chitin and Chitosan.	32
5.3.1	FTIR Result of <i>P. pelagicus</i>	33
5.3.2	FTIR Result of <i>P. sanguinolentus</i>	34
5.5.1	SEM Image of <i>P. pelagicus</i>	36
5.5.2	SEM Image of <i>P. sanguinolentus</i>	36
5.5.3	Result of Antimicrobial activity	37

LIST OF TABLES

TABLE NO	TITLE	PAGE NO
4.1	Biochemical value of Blue crab (<i>P. pelagicus</i>)	21
4.2	Biochemical value of Three spotted crab (<i>P. sanguinolentus</i>)	21
5.1	Yield of Chitin and Chitosan (%)	32

CHAPTER 1

INTRODUCTION

Marine invertebrates are being widely used as food and feed supplement around the world. Shell fish provide valuable nutritional resources for humans, including high-quality protein concentrations and a variety of minerals in valuable forms (USDA, 2015). While some species are found in a sizable portion of the freshwater fauna and terrestrial ecosystems most species are still found in the waters, from the abyssal zone to the coastline. (lindberg, 2008). One of the valued seafood products that is in high demand in India's export and domestic markets is marine crabs. At the international level, nations like China, France, and Indonesia According to (Radhakrishnan, 2003), the United States, Japan, the Philippines, Spain, and Thailand regard the crab types as having the greatest economic significance. A crab's broad, flattened body is protected by a carapace, or shell. Members of the class Crustacea, order Decapoda, and phylum Arthropoda comprise crabs. The order Decapoda contains two sub-orders: (a) *Natantia*, which are swimmers, and (b) *Reptantia*, which are crawlers. Crabs, lobsters, and hermit crabs are members of the reptilian group. Typically, crabs are short-tailed decapod crustaceans that are found all throughout the world, but especially in tropical and semi-tropical areas. The greatest places in India to go crab fishing are the coasts of Tamil Nadu, Kerala, Karnataka, and, to a lesser extent, Maharashtra and Gujarat. The *Portunidae* family is home to the majority of marine crabs that may be found all across the Indian coast. India's crab industry is expanding quickly; in 2013–2014, 44,586 t of crabs were reported to have been landed along the coast. India received 37.27 lakh tones of marine fish landings in 2019–20. The majority of human consumption occurs from the claws or legs of hard-shell crabs, whereas very few soft-shell crabs are consumed in their entirety (Aasham *et al.*, 2006). Crab is a sort of seafood that is served in many nations, particularly in North America and Europe. In addition to having excellent medical, socioeconomic, and cultural benefits, crab is a rich source of protein (Large-yusty *et al.*, 2011). It is said that eating crab meat nourishes the body, improving mental clarity, lowering inflammation, bolstering bone density, enhancing immunity, promoting blood flow, and cleansing the body (FAO,

2010). According to (Barber, 2013), it is essential for the synthesis of red and white blood cells, decreasing blood pressure, shielding the body from radiation harm, and helping the body eliminate heavy metals. Crab shells are bio-sorbent, crabs can be used as indicators of aquatic pollution in their habitat (An, 2001). Because of its great nutritional value, crab meat is the most sought-after seafood among shellfish products worldwide. Recently, South Asian nations have seen an increase in the commercial demand for horseshoe crab in the biomedical sector (Krisfalusi-Gannon *et al.*, 2018). Moreover, it has been found that crab flesh has a high content of omega-3 polyunsaturated fatty acids, which promote improved cardiovascular health, but a low fat content. (Islam *et al.*, 2017). Overall, there are a number of health benefits that come with eating crab meat, including significant amounts of digestible proteins, long-chain polyunsaturated fatty acids, bioactive peptide, astaxanthin and other carotenoids, minerals (copper, zinc, inorganic phosphate, salt, potassium, iron, and iodine), vitamins, especially vitamin B12, and other substances (Gopakumar, 2017). The edible crabs (*Cancer pagurus*), mud crabs (*Scylla serrata*), snow crabs (*Chionoecetes opilio*), flower crabs (*Portunus pelagicus*), blue crabs (*Callinectes sapidus*), and dungeness crabs (*Metacarcinus magister*) are the most significant crab species along the world, these species are valuable food sources in both domestic and international open markets (Zafar *et al.*, 2013). Crabs are the third most popular edible marine crustacean, behind lobster and shrimp, because of their value as a delicacy and the fishery they support (Heu *et al.*, 2003). Because of their size, meat content, and distinct flavor, crabs are highly sought-after as a delicacy and high-quality food, which has led to their high prices in both domestic and foreign markets (Kathirvel, 1993).

The most adaptable macromolecules in biological systems, proteins are essential to every aspect of biological function. Twenty distinct amino acids with considerable nutritional significance are found in the proteins found in crab tissue. Among these amino acids are the following: aspartic acid, valine, methionine, arginine, isoleucine, leucine, phenylalanine, lysine, histidine, and tryptophan as Essential amino acids (EAA) and glutamic acid, cysteine, tyrosine, alanine, asparagine, proline, serine, and taurine as Non-Essential amino acids (NEAA). The essential amino acid (EAA) concentration of seafood items, particularly marine crabs, is largely responsible for their

flavor, nutritional value, and health advantages (Chen *et al.*, 2007).and it provides vital nutrients for human development as well as bodily processes like physiology, biochemistry, and immunity (Maria *et al.*, 2007).Crab meat provides primarily with protein and amino acids, making up about 80% of the total protein in the body, according to (Chen *et al.*, 2007). They generate motion, convey nerve signals, function as a stimulant, transport and retain other substances like oxygen, provide mechanical and immunological assistance, and control growth and differentiation. (Berge, 2002).According to (Strausburg, 2000) Actin, Tropomyosin, Troponin, and Nebulin. Myosin makes up the majority of the thick filaments, though fitting is frequently connected to them as well. The protein bands in the 18–20 KDa range observed in the gills and wash supernatant were shown to be linked with myosin light chain and troponin (Thorarinsdottiret *et al.*, 2002) The myosin heavy chain was most likely the band at 190KDa (Thorarinsdottiret *et al.*, 2002). In addition, among many other invertebrates, crabs are regarded as a vital product of the shellfish industry (nalan *et al.*, 2003).

Lipids are an important biochemical component of marine food webs because they offer a concentrated source of energy that is rich in carbon. Moreover, lipids are currently frequently investigated in ecological research as biomarkers and as instruments to comprehend large-scale oceanic phenomena (Budge *et al.*, 2006). The nutritional advantages of eating crustaceans include low levels of fat and cholesterol in the muscle, as well as a high quantity of protein, necessary minerals, fatty acids, and amino acids (Sara Barrento *et al.*, 2009). Additionally essential to human health are marine lipids, and declining seafood stocks (FAO, 2010) More and more research is supporting the eating of crustaceans, even if it is generally not advised due to allergic reactions or the purported high cholesterol content (Nunes *et al.*, 2003). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), two omega-3 polyunsaturated fatty acids (PUFAs), were thought to provide a host of health benefits for humans. Consuming PUFA lowers the danger of cancer and heart attack in the coronary heart which has (Mahaffey *et al.*, 2003) antiatherogenic and antithrombotic properties as well as the ability to control hypertension and rheumatoid arthritis. Moreover, it lowers the chance of developing diabetes and stops heart arrhythmias. Additionally EPA and DHA are the building blocks of several metabolites that are potent

lipid mediators and may be useful in the management or avoidance of certain illnesses. by many researchers (Serhan *et al.*, 2008).

Animals primarily use carbohydrates, a class of organic substances that includes sugars, starches, and fiber, as a source of energy. Chemically speaking, carbohydrates are higher polyhydric alcohol derivatives such as aldehydes or ketone compounds. The carbohydrates found in fisheries products are made up mostly of glycogen and have little dietary fiber. Additionally, they contain trace amounts of other mono- and disaccharides, sucrose, fructose, and glucose (Okuzumi and Fujii, 2000). They are a crucial source of energy for the diet of humans. Carbohydrates are regarded as an essential food source in high-cost, staple diets. In animal tissues, the proportion of carbohydrates was lower than that of other nutrients including proteins and lipids (Soundarapandian *et al.*, 2013). It is well known that seafood has extremely little carbohydrate content (Elegbede *et al.*, 2013). This may be because of the natural aquatic food web's abundance of lipids and protein and shortage of carbs, and most likely because of this, aquatic animals frequently use protein as a source of energy molecule.

The Indo-Pacific, including off the coasts Indonesia, Malaysia, Cambodia, Thailand, the Philippines, and Vietnam; It is a significant fishery in the United States (Chronin, 2007). Europe first encountered the blue crab at the beginning of the century (Nehring, 2011). Understanding the differences in the cuticle induced by variables including gender, growth, sample position on the carapace, and molt cycle is essential to using cuticle features as taxonomic traits in both fossil and modern decapods. This understanding is essential for the identification of taxonomically significant characteristics (Waugh *et al.*, 2003). From the coast of Canada to northern Argentina, the blue Swimmer crab (*Portunus pelagicus*) is the valuable fishery. The Mediterranean is another region where it has been found. Over the preceding few decades, the projected yearly global production of blue crabs was 300,000 tan. A traditional delicacy, blue crab meat commands a high price on the market, with an average cost of 35.8405 dollars per lb. It is categorized as “jumbo lump,” “backfin,” “special,” or “clamp” and pertains to the crab's muscular tissue (PAOLISSO, 2007). While the season, age, and eating habits can all affect how much blue crab flesh is ingested, in general, the meat

has 76.86% moisture content, 19-14% protein content, and 0.5-0.8% fat content. Additionally, the meat that comes from the crab is high in calcium, phosphorus, magnesium, sodium, potassium, manganese, zinc, and iron, and it has a negligible amount of carbohydrates. Eating crab has several nutritional advantages, such as its high protein content, which is necessary for macro and trace elements, fatty acids, and amino acids, as well as its low fat and cholesterol content, which is concentrated in the crab muscle. (Barranto *et al.*, 2010).

Spotted crab (*Portunus sanguinolentus*) are a traditional edible species of crab that play a major role in India's fishing economy (Radhakrishnan *et al.*, 2007). Understanding nutrients is crucial, but with the rising demand for food, even more so is protecting it from deterioration. Food safety and standards of identification requirements are even increasingly crucial because this trend suggests that fishery products are highly mobile over the world, necessitating the establishment of strict traceability systems to monitor the commodity's transit from harvest to consumers. Developed countries import almost 75% of the seafood traded internationally, whereas underdeveloped countries export 50% of that total. As a result, concerns about food safety related to seafood are now worldwide in scope rather than local or limited to a certain area (Scallen *et al.*, 2011). One of the fisheries resources in the South East Asian waters is portunid crabs, of which the swimming varieties, such as the blue swimming or flower crab (*P. pelagicus*) and the three spot crab (*P. sanguinolentus*), are highly valuable commercially in addition to mud crabs. Most of the oceans in India, Bangladesh, Southeast Asia, and the Arab world are heavily fished for blue crabs. The coasts of Tamil Nadu, Kerala, Karnataka, and, to a lesser extent, Gujarat and Maharashtra in India have the best potential. The majority of captures in Tamil Nadu come from the Gulf of Mannar and Palk Bay, Nagapattinam, and Puducherry, which accounts for over 90% of all crab landings along the east coast (Rao *et al.*, 1973).

A great deal of focus has been placed on seafood and its byproducts as a vital source of nutrients for human nutrition. According to (Adeye *et al.*, 2002) *P. sanguinolentus* and *P. pelagicus* have high levels of amino acids that aid in the management of a number of inflammatory, rheumatoid arthritis, Crohn's, and cardiovascular disorders. A notable quantity of calcium, magnesium, and phosphorus

can be found in crabs' hard shells, which could be utilized to make pharmaceuticals and biofertilizer. (Priya *et al.*, 2015) shown that the essential amino acid compositions of *P. sanguinolentus*' meat protein are well balanced, with high concentrations of glycine, arginine, glutamine, alanine, and proline. Additionally, it has been found that the total amount of essential and non-essential amino acids is comparable to that of spider crabs (Marques *et al.*, 2010), somewhat greater than that of blue swimmer crabs (Wu *et al.*, 2010) and double that of Chinese mitten crabs that are captured in the wild (Priya *et al.*, 2015).

1.1 CHITOSAN EXTRACTION AND ANTIMICROBIAL ACTIVITY

Chitosan, a polysaccharide derived from the chitin found in the exoskeletons of different crustaceans, can be obtained from waste materials of shrimp and crabs. A number of studies conducted recently have highlighted the potential use of insects, mushrooms, coral, and the eggs of resting crustaceans as substitute sources of chitin. Recently, almost 20,000 species of Orthoptera have been identified; yet, in terms of chitin extraction, these organisms have received very little attention (Davies *et al.*, 2013). An acid solution is used for demineralization, an alkaline solution is used for deproteinization, and a concentrated alkaline solution is used for deacetylation (DA).

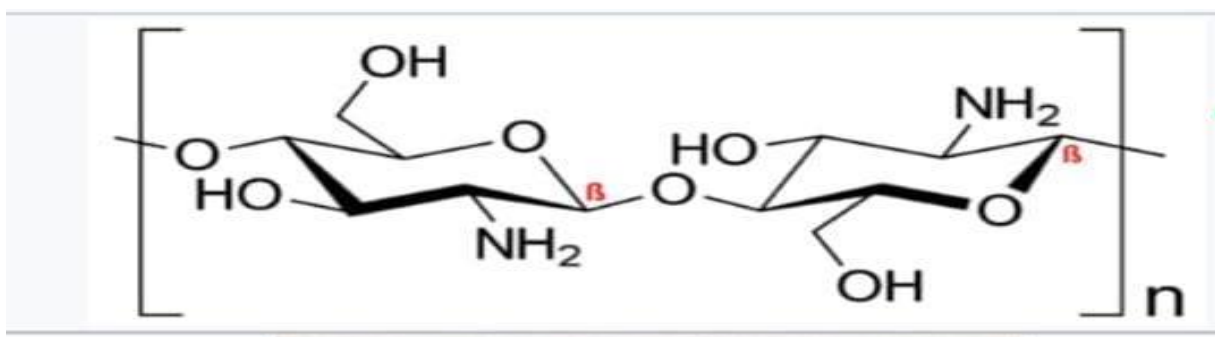


Fig 1.1: Chitosan Structure

Nevertheless, chitosan is produced by eliminating sufficient acetyl groups (CH₃-CO), leaving behind a full amino group (-NH₂) in addition to the (OH) group, both of which are active sites in numerous chemical interactions. Chitin, on the other

hand, is composed of a linear chain of acetyl glucosamine groups (Cacciari *et al.*, 2015). Chitosan is organic, non-toxic, biodegradable, and biocompatible polymer that possesses antibacterial properties. It finds extensive use in various domains, including membranes, agricultural (fertilizers, insecticides, and pesticides), cosmetic (toothpaste, body creams, and shampoos), biomedical and pharmaceutical (tissue engineering—artificial skin, wound healing, drug delivery), and food processing (packaging, juice clarification, and protein recovery) (Benhabiles *et al.*, 2017). fuel cells, textile, and paper industry (Vikele, 2017). Although As a weak base, chitosan is soluble in diluted acidic aqueous solutions (pH less than 6.5) but insoluble in organic solvents and water. and change glucosamine units into soluble forms of protonated amines (R-NH₃) (Kunjachan & Jose, 2010). Because of its distinct size and high surface-to-volume ratio, sorbent particle size that is, the synthesis of chitosan at the nanoscale can regulate the ability of chitosan or even natural polymers to adsorb (Agarwal *et al.*, 2015).

Chitin, which combines with proteins to form a chitin protein complex, makes up the waste material found in crab shells. Different materials make up fish scales, shrimp, and crab shells, including proteins (15–50%), minerals (30–50%), and chitin (15–30%). (Younes *et al.*, 2014). It has been discovered that the chitosan from *Portunus sanguinolentus* exhibits antibacterial and antioxidant qualities (Kaya *et al.*, 2016) amino and hydroxyl groups, which have the ability to scavenge free radicals and help shield cells and tissues from oxidative damage are primarily responsible for the antioxidant action of this substance. Chitin and chitosan have so many uses, it is imperative that new resources be developed (Liu *et al.*, 2012). Since It is well known that infections, both bacterial and fungal, have evolved defense mechanisms against antibiotics. it is imperative to create more potent antimicrobial agents as a backup. Chitosan has been demonstrated in the literature to exhibit antibacterial action against a range of bacteria, fungi, and yeasts as an alternative antimicrobial agent (Uchida *et al.*, 1989). Because of their antibacterial properties, they are employed as coating agents on a variety of food wrappers to preserve food. 8. Because they are non-toxic and non-allergic, they can be employed in wound care and other biomedical applications. The Present study aim to ascertain the biochemical makeup (moisture,

protein, carbohydrate, and lipid content) of selective Marine Crab. And Assist antimicrobial activity from the Crab Chitosan

CHAPTER 2

LITRETURE SURVEY

Zotti *et al.*,2016 The analysis's goal was to profile each of the three crab species' nutritional value and chemical traits. The native warty crab (*Eriphia verrucosa*) and the commercially edible crab (*Cancer pagurus*) were compared with the proximate composition and element contents of the claw muscle tissue of Atlantic blue crabs (*Callinectes sapidus*). The element fingerprints revealed notable variations between specifics, while negligible differences were observed in the amount of moisture and ash. The protein content of blue crabs was noticeably lower than that of the other Two species, although the carbon content was found to be lower than that of just the warty crab. Ba, Cr, Cu, Li, Mn, Ni, and Pb were among the micro-elements with incredibly low quantities and minimal differences between species. There were notable variations between species for Na, Sr, V, Ba, Cd, and Zn; specifically, zinc and cadmium. was Blue crabs are distinguished by concentrations that are noticeably lower than those of the other two species.

Samuel *et al.*,2009 Crabs are the third most valuable edible marine crustacean in India, after lobster and shrimp, which are ranked first and second respectively, due to their status as a luxury food and the importance of the fisheries they sustain. The genus *Scylla* is the most important group of crabs for commerce, followed by (*Portunus sp*, *P. pelagicus* and *P. sanguinolentus*) The goal of the current study is to examine the fisheries Possibility of commercial significant *portunid* crab along the Parangipettai coast. It seems that *P. sanguinolentus* breeds.

year-round, according to the data. The livelihood along the coast was severely damaged by the Killer waves. The area of research is situated along the coast of Tamil Nadu, specifically in the districts of Nagapattinam and Cuddalore, where fishermen frequently lose their boats and gear. The berried crabs were observed all year round, with two seasonal peaks in February and May of 2004 and August and December of 2004 respectively, during the first year. On the other hand, the second year's peak occurs from August 2005 to December 2005, with the second peak occurring between

May and June 2005. In the first year, from February 2004 to December 2004, The landings of ovigerous female crabs were noted. at 5,193.7 kg (5.19 tons), while in the second year, from May 2005 to January 2006, the landings totaled 4,337.6 kg (4.33 tons).

Rangasamy *et al.*, 2024 They looked at the mineral concentrations, fatty acid profile, and proximate composition of the swimming crabs, *Portunus sanguinolentus* and *Portunus pelagicus*. *P. sanguinolentus* contains 26.52% protein and 10.93% fat. *P. pelagicus* contained 16.62% protein and 9.93% fat. Out of the fifteen fatty acids found in crab oil. With a share of 31.3%) and 26.59% of the overall fatty acid profile, PUFAs predominate. The greatest component was methyl stearate (11.88% and 11.69%), which was followed by docosahexaenoic acid (8.93% and 8.85%), in that order. The coastal crabs of Rameswaram are rich in minerals, including calcium, magnesium, sodium, potassium, manganese, and zinc. The two species of Rameswaram seashore genus *Portunus* crabs are clearly more expensive than sea food according to this study.

Zakzok *et al.*, 2022 One of the most significant and profitable crabs in the world, especially for Egyptian fisheries, is *Callinectes sapidus*. In particular, condition factor, biochemical composition, and morphometric and weight correlations of the blue crab population in Manzala Lake, Mediterranean Sea, Egypt, were the baseline data that this study was conducted to give. Samples were gathered from March 2019 to February 2020, and seasonal analysis was done on the data. In comparison to females, male crabs were noticeably bulkier and more numerous. Just 1.12% of specimens exhibited carapace widths between 30 and 49.9 mm, whereas the majority (69%) had widths between 70 and 109.9 mm. In *Portunus pelagicus*, the overall sex ratio was 1.41:1, favoring males. Hepatosomatic index (HSI) and gonadosomatic index (GSI) exhibited a moderate to poor connection ($r^2 = 0.32$ in females and 0.52 in males). Nonetheless, there was no statistically significant variation in the muscle's biochemical makeup between the sexes blue crabs ($p > 0.05$). Additionally, a comparison between the current results and those from various locations is provided.

Sudhakar *et al.*, 2011 The biochemical makeup of ten economically significant crabs (*Scylla serrata*, *S. transquebarica*, *Portunus pelagicus*, *P. sanguinolentus*, *P.*

gladiator, *Charybdis feriata*, *C. lucifera*, *C. natator*, *C. granulata*, and *Podophthalmus vigil*) was examined in the current study. The protein contents were discovered to be following in the order (*S.tranquebarica* >*S.serrata* >*P.sanguinolentus* >*P.pelagicus* >*C.natator*>*C.lucifera* >*C.feriata* >*P.gladiator* >*P.vigil* >*C.granulata*).Maximum (42.30%) protein content was identified in *S.tranquebarica* and minimum recorded in *C.granulata* (17.90%).It was discovered that *P. gladiator* (1.46%) had a lower value of carbohydrates than *S. serrata* (2.17%). In the current investigation, *P. gladiator* had the lowest lipid content (0.97%) and *S. tranquebarica* had the highest (1.48%). Therefore, in terms of nutritional value, mud crabs are deemed superior to blue swimming crabs. It was discovered that *P. gladiator* (1.46%) had a lower value of carbohydrates than *S. serrata* (2.17%). In the current investigation, *P. gladiator* had the lowest lipid content (0.97%) and *S. tranquebarica* had the highest (1.48%). Therefore, in terms of nutritional value, mud crabs are deemed superior to blue swimming crabs.

2.1 CHITOSAN EXTRACTION AND ANTI MICROBIAL ACTIVITY

Pradhan *et al.*, 2024 Chitosan is usually extracted chemically using a number of procedures, including alkaline deproteinization, acidic demineralization, chemical agent bleaching, and alkaline deacetylation. Each of these procedures has a major impact on the physicochemical properties of the resulting chitin and chitosan. But this traditional extraction process also produces chemical waste, therefore wastewater needs to be neutralized and purified. Crab shells are an abundant waste product from the seafood sector that is easily accessible. In this work, the FESEM images of commercial and crab chitosan was analyzed. It was found that throughout the N deacetylation process, a clearly organized microfibrillar crystalline structure was generated in the crab chitosan.

Mary *et al.*, 2023 Crab exoskeletons were used to make chitosan by undergoing a number of procedures including demineralization, deproteination, and deacetylation. Here, the physicochemical characteristics were examined. The crab shell's moisture content, solubility, ash content, degree of deacetylation, water absorption capability,

and water binding and fat binding capacity were all measured. Chitosan exhibited the following capacities: $516 \pm 12.48\%$ for water absorption, $500 \pm 16.32\%$ for water binding, and $406 \pm 16.99\%$ for fat binding. Chitosan had a deacetylation level of $83.16 \pm 0.47\%$. Chitosan had a moisture content of $8.92 \pm 0.27\%$. The chitosan's solubility was 73.86 ± 1.35 percent. The chitosan's ash content was $1.42 \pm 0.09\%$. This study demonstrates how to produce high-quality chitosan using crab (*Portunus sanguinolentus*) shells.

Kaya *et al.*, 2016 Chitin was extracted from the shell of *Portunus pelagicus* using a traditional chemical process. The crab shells' dry weight was and the chitin content was found to be 12.1%. A 76% yield of chitosan was reported overall. It was discovered that 82.5% of the chitosan had a deacetylation degree of The results of FTIR, TGA, and XRD investigations verified that the obtained chitin was in the α - form As a result, chitin exhibited a in result number, while chitosan revealed a value of 306 °C in the same regard. Using SEM, precise data regarding the makeup of pores and nanofibers was acquired. The disc diffusion method used to test the antimicrobial activity revealed that the inhibition zone diameters of the chitosan from *C. sapidus* varied between 15.28 and 20.21 mm for human bacterial pathogens, between 15.51 and 16.25 mm for fungal pathogens, and between 14.22 and 15.75 mm for fish bacterial pathogens. The chitosan derived from *C. sapidus* exhibited MBC and MFC values ranging from 0.16 to 2.50 mg/ml. The activity of ferric ions reducing power and DPPH results, which were utilized to assess the chitosan's antioxidant activity, were determined to be, respectively, IC₅₀: 5.99 and EC₅₀: 6.16.

Bolgen *et al.*, 2016 chitin is predominantly found in the exoskeleton of many crustaceans, chitosan is a polymer that occurs naturally and is biocompatible, biodegradable, nontoxic, antibacterial, antioxidant, and antifungal In this study, chitin was chemically removed from the blue crab's exoskeleton (*Portunus pelagicus*). Samples of *Portunus pelagicus*, which are leftovers from food consumption, were gathered at a market. To extract chitin, the samples underwent decolorization, deproteinization, and demineralization processes. Isolated chitin was deacetylated at high temperatures to produce chitosan. FTIR measurements were used to evaluate the chemical composition of the crab shell, extracted chitin, and chitosan. Chitosan cryogel

scaffolds were created via a crosslinking procedure with varying ratios of glutaraldehyde (1, 3, and 6%, v/v) as the crosslinker, while the chitosan content remained constant at 1% w/v. FTIR was used to analyze the scaffolds' chemical makeup. Additionally, scaffolds' ability to absorb water has been established. All things consider, the data implied that the cryogels made of chitosan that have been defined may serve as viable scaffolds for tissue engineering applications.

CHAPTER 3

AIM AND OBJECTIVE

3.1 AIM:

- To Determine the moisture loss of selected marine crabs.
- To Estimate total protein content present in two different crab species.
- To Assess carbohydrate content of two different crab species.
- To Determine fat content.
- To Extraction of chitosan present in crab shell and characterization of chitosan.
- To Study the Antimicrobial activity of crab chitosan.

3.2 OBJECTIVE:

- Determination of moisture content.
- Estimation of protein content.
- Carbohydrate estimation.
- Determination of fat content.
- Chitosan extraction from crab shell.
- Characterization of chitosan
- Antimicrobial activity.

CHAPTER 4

MATERIALS AND METHODS

4.1 SAMPLE COLLECTION AND DISSECTION

The Crab Species (*Portunus pelagicus* and *Portunus sanguinolentus*) collected from southeast coast of Pudukkottai, Tamilnadu, India. Specimens were collected and then brought to the laboratory alive in chilled containers. The Species were Dissected by using dissection kid, the both muscle tissue were taken as a sample for proximate analysis Where the crab Shells were taken as for extraction of chitosan. Every specimen that was gathered yielded two samples of muscle tissue were weighed and Assess Biochemical composition such as, Moisture, Protein, Carbohydrate, and Fat content and the Crab shells were dried and grind it for Chitosan extraction.

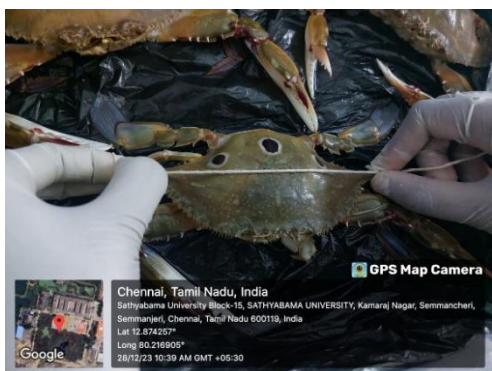


Fig 4.1.1: *Portunus sanguinolentus*



Fig 4.1.2: *Portunus pelagicus*

4.2 PROXIMATE ANALYSIS

The proximate analysis done by estimation of Moisture, Protein, Carbohydrate and overall Fat content present in selected marine crabs (*Portunus pelagicus* and *Portunus sanguinolentus*).

4.2.1 Determination of moisture content by AOAC method

Moisture content was Performed by AOAC Method (AOAC (2010). The Moisture content was determined were A Metal Moisture dish with a little acid – washed sand in it is placed in a hot air oven at 105⁰c and left overnight. After drying the moisture content were measured by using weighing balance.

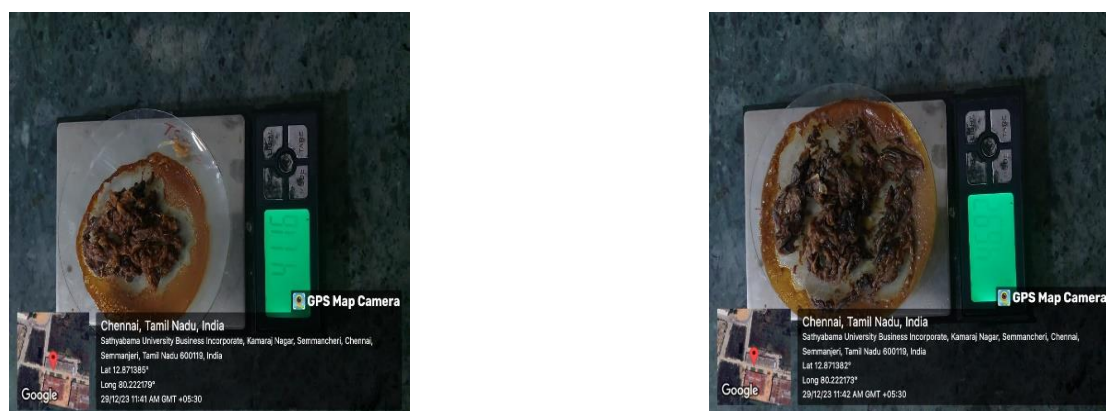


Fig 4.2.1: Moisture Estimation of *P. pelagicus* (Blue Swimmer Crab)

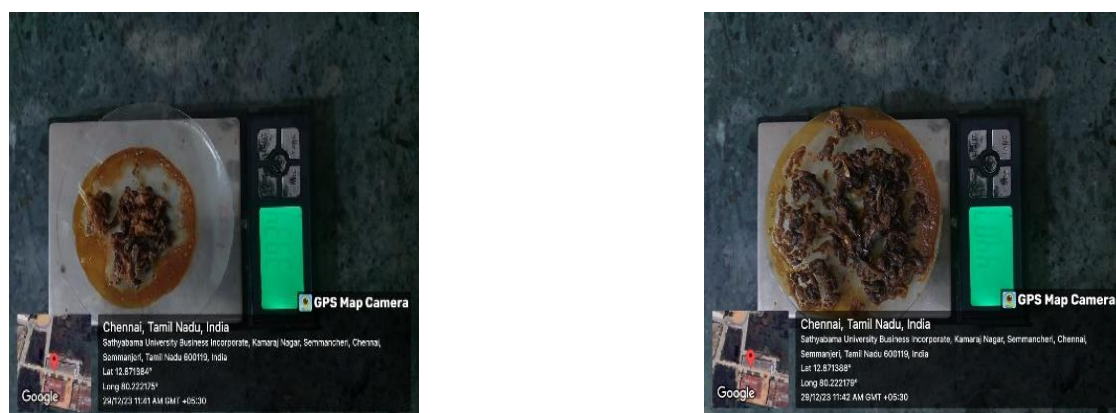


Fig 4.2.2: Moisture Estimation of *P. sanguinolentus* (Three Spotted Crab)

4.2.2 Estimation of carbohydrate by anthrone method

1. Anthrone Reagent

Dissolved 100mg of anthrone in 50 ml of concentrated H_2SO_4 (Prepare fresh before use-Light sensitivity).

2. Standard solution

(a) 0.5g of muscle powder(sample) made upto 0.5 ml of distilled water (100mg/100ml).

(b) Diluted 10 ml of stock standard solution into 100 ml with distilled water in a volumetric flask (10mg/100 ml).

0.5 g of working standard solution in three different test tubes were taken as s_1 - s_3 . And 0.5 ml of distilled water were added in each one of the test tubes. after that kept in it on vortex of 15 mins for mixing. And then 4ml of anthrone reagent were inserted into each test tube

Mixed the contents well and kept the test tubes in water bath at 100°C for 10 mins. Seen Colour changes and Cooled the test tubes in ambient temperature and measured the absorbance within a photoelectric colorimeter at 620 nm.

Calculation:

Absorbance of test

Amount of test = _____

Absorbance of Standard X Amount of Sample.



**Fig 4.2.3: (a) Carbohydrate Estimation
Of *P. sanguinolentus***



**Fig 4.2.4: (b) Carbohydrate
Estimation of *P. pelagicus***



**Fig 4.2: Result were calculated
using microwell plate reader for
both (a) and (b)**

4.2.3 Determination of protein by lowry's method

1. Analytical Reagent:

(a) 0.5 g of copper sulfate (CuSO_4) in 50 ml of distilled water

(b) 2% of Sodium potassium tartrate in 50 ml of distilled water

(c) 2% of Alkaline Sodium carbonate in 0.1N of NaOH

Mix the solution A (50 ml)

Solution B (0.5 ml)

Solution C (1 ml) made up to Analytical reagent.

2. Follin's Reagent:

25 ml of Follin's Reagent dissolved in 25 ml of distilled water.

0.5g of triplicate sample into a series of labeled test tubes S_1 - S_3 . The blank is a tube filled with one milliliter of distilled water.

And then 2.5 ml of analytical reagent were added in each one of the test tubes. incubated for ten minutes at room temperature.

0.5 ml of Follin's reagent were added into the test tubes. Mixed the contents of the tubes by vortex the tubes kept it in incubation at room temperature for 30 mins. Read the absorbance at 720 nm against blank. By plotting the protein amount along the X-axis and the absorbance at 720 nm along the Y-axis, one can plot the standard curve. determined how much protein was present in the sample.

Calculation:

Amount of test = Absorbance of test $\times$ Amount of standard.

Absorbance of standard.



Fig 4.2.6: (a) Analytical Reagent



Fig 4.2.7: (b) Follin's Reagent



Fig 4.2.8: (c) Protein Estimation

4.2.4 Determination of fat by bligh and dyer's method

0.5g freshly prepared tissue sample were taken and homogenized with 1ml of chloroform and 2ml of methanol and added 1ml of chloroform and blended for 30 mins. 1ml of chloroform blended into 0.8 ml of NaCl. blended it again. Next the watt man filter paper was used to filtered the liquid phase and two layers of methanol and chloroform were formed in the test tubes. the topmost stratum of methanol was discarded and the layer underneath of chloroform would take and kept into aluminium dish of thin coating. Heat it in metal hot plate oven for 15 mins. Finally measure the total fat content by used weigh b

Calculation:

Weight of empty dish (g) = w_1 ;

Weight of dish and extracted fat (g) = W_2

Weight of sample = S% Grude fat = $(W_2 - W_1) \times 100/S$

Table 4.1: Biochemical value of Blue Swimmer Crab (*Portunus Pelagicus*)

MOISTURE	PROTEIN	CARBOHYDRATE	FAT
80.8%	18.12%	0.65%	2.19%

Table 4.2: Biochemical value of Three spotted Crab (*Portunus Sanguinolentus*)

MOISTURE	PROTEIN	CARBOHYDRATE	FAT
84.6%	16.3%	1.12%	1.92%

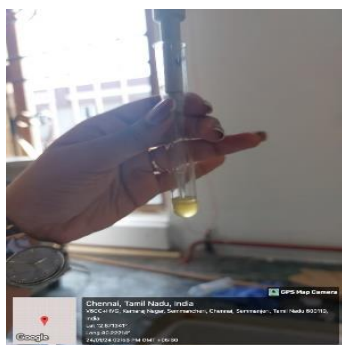


Fig 4.2.9: Chloroform methanol separation

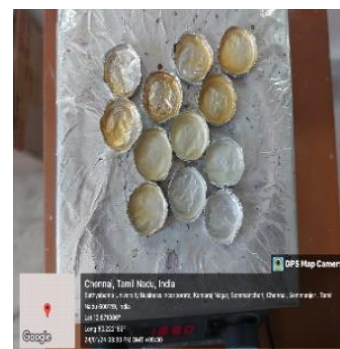


Fig 4.2.10: Chloroform into Aluminium dish for total fat Estimation

4.3 CHITOSAN EXTRACTION FROM CRAB SHELL

The Crab Shells were dried in the solar drier and grind it made it of powder for extraction of chitosan. The preparation technique for chitin and chitosan involved the use of analytical grade hydrochloric acid and sodium hydroxide. Additionally, distilled water was used to wash the sample and make the chemical solution at the appropriate concentration.

Then the *Portunus Sanguinolentus* and *Portunus pelagicus* Shell powder were processed as follows to create chitosan powder: deproteinization, demineralization, decolorization, and deacetylation.

4.3.1 Deprotenization

In order to extract the protein from the crab species shell waste from the carotenoid extraction process, 4.0% of NaOH solution was added, and the crushed shell to solution ratio was 1:20 (W/V). The mixture was continuously stirred for two hours at 90° c.

After filtering the shell powder the filtrates had been rinsed after 30 minutes of using tap water, or until the pH reached a neutral level (7). The deproteinized shells were then baked for 24 hours at 60 degrees Celsius.

4.3.2 Demineralization

In order to remove the mineral content, the deproteinized crab shell powder was demineralized with 2.5 percent (w/v) of HCl for six hours at room temperature. The ground shell to solution ratio was 1:20 (w/v).

After filtering, the samples were rinsed with tap water for 30 minutes, or until the pH reached a neutral seven. After that, the demineralized shell powder was dried for 24 hours at 60 degrees Celsius in an oven.

4.3.3 Decolouration and dewatering

The samples were decolored by treating them with acetone at a ratio of 1:10 (w/v) for 10 minutes, drying them for two hours at room temperature, and then eliminating any remaining acetone residue.

The decolorized shell powder was rinsed under running tap water, filtered, and baked in an oven at 60 degrees Celsius for 24 hours to extract crab chitin.

4.3.4 Deacetylation of chitin

After chitin was obtained, it was treated for two hours at 105 degrees Celsius with 50 percent (w/v) aqueous NaOH at a chitin to solution ratio of 1:15 (w/v).

Then, in order to extract the chitosan, After filtering and washing the chitin in deionized water, the pH was brought down to a neutral 7.

After that, the chitosan was dried in an oven set at 60 degrees Celsius for a whole day. Ultimately, the substance was ground up and sealed as chitosan.



Fig 4.3.1: Before Deprotenization



Fig 4.3.2: After Deprotenization



Fig 4.3.3: Before Demineralization



Fig 4.3.4: After Demineralization



Fig 4.3.5: Before Decolourization and Dewatering



Fig 4.3.6: After Decolourization



Fig 4.3.7: Before Deacetylation

4.4 ANTIMICROBIAL ACTIVITY OF CHITOSAN

The chitosan solution was made by dissolving the chitosan in one percent acetic acid. After preparing and sterilizing nutrient agar (NA) medium, the antibacterial activity

of this solution was assessed for antimicrobial activity. The surface of sterile NA plates was swabbed with the culture of pathogenic bacteria. The antibacterial activity was assessed using the Agar Well Diffusion Method. Using a sterile micropipette tip, 100 mg of chitosan solution was applied to the 6 mm well that was punched over the (NA) plates. At 370⁰ C, these agar plates were incubated for a whole day. Following the incubation time, the zone of inhibition surrounding the wells was measured and examined.



Fig 4.4.1: *Aeromonas hydrophila*



Fig 4.4.2: Nutrient Agar

Loaded into the Nutrient Broth

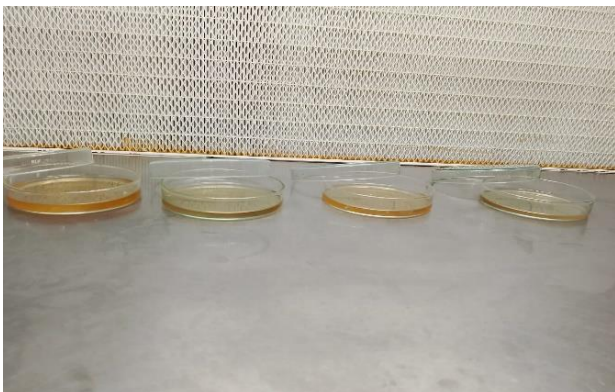


Fig 4.4.3: Solidified Nutrient Agar

Plate

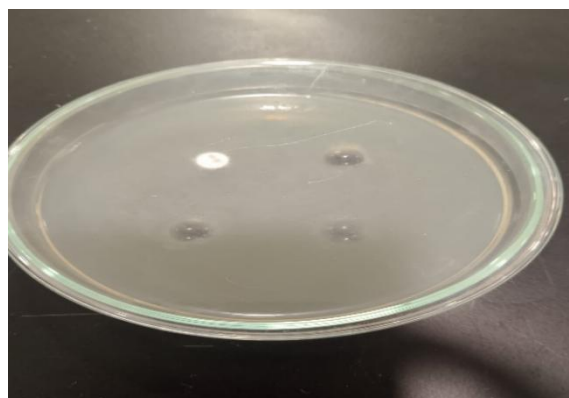


Fig 4.4.4: Agar Well Diffusion

method

4.5 CHARACTERIZATION OF CRAB CHITOSAN

Chitin and chitosan yield: The weight of the extracted chitin was divided by the initial dry weight of the crab shell to determine the percentage of the chitin yield, and the weight of the produced chitosan was divided by the dry chitin weight prior to deacetylation to determine the percentage of the chitosan yield. Following is the calculation of yields.

$$\text{Yield of chitin (\%)} = [\text{Produced chitin (g)}/\text{Crab shells (g)}] \times 100$$

$$\text{Yield of chitosan (\%)} = [\text{Extracted chitosan (g)}/\text{Chitin (g)}] \times 100$$

4.5.1 FTIR Analysis

The substance underwent Fourier transform infrared (FTIR) examination across a 400–4000 cm^{-1} frequency range using a Perkin Elmer spectrum RX1 analyzer. The level of deacetylation of the chitosan was determined.

4.5.2 Solubility test

A tube for a centrifuge containing (0.1 g in triplicate) of the chitosan that was generated was filled with 10 mL of a 1% acetic acid solution. For thirty minutes, the

sample was centrifuged at 10,000 rpm. Following the removal of the supernatant, the remaining undissolved chitosan was centrifuged at 6,000 rpm after being cleaned with 25 mL of distilled water. The liquid supernatant was emptied, and the material that hadn't dissolved was oven-dried for 24 hours at 60 degrees Celsius. Finally, the dried solid's mass was weighed in order to calculate its solubility percentage.

4.5.3 Scanning Electron Microscopy (SEM) Analysis

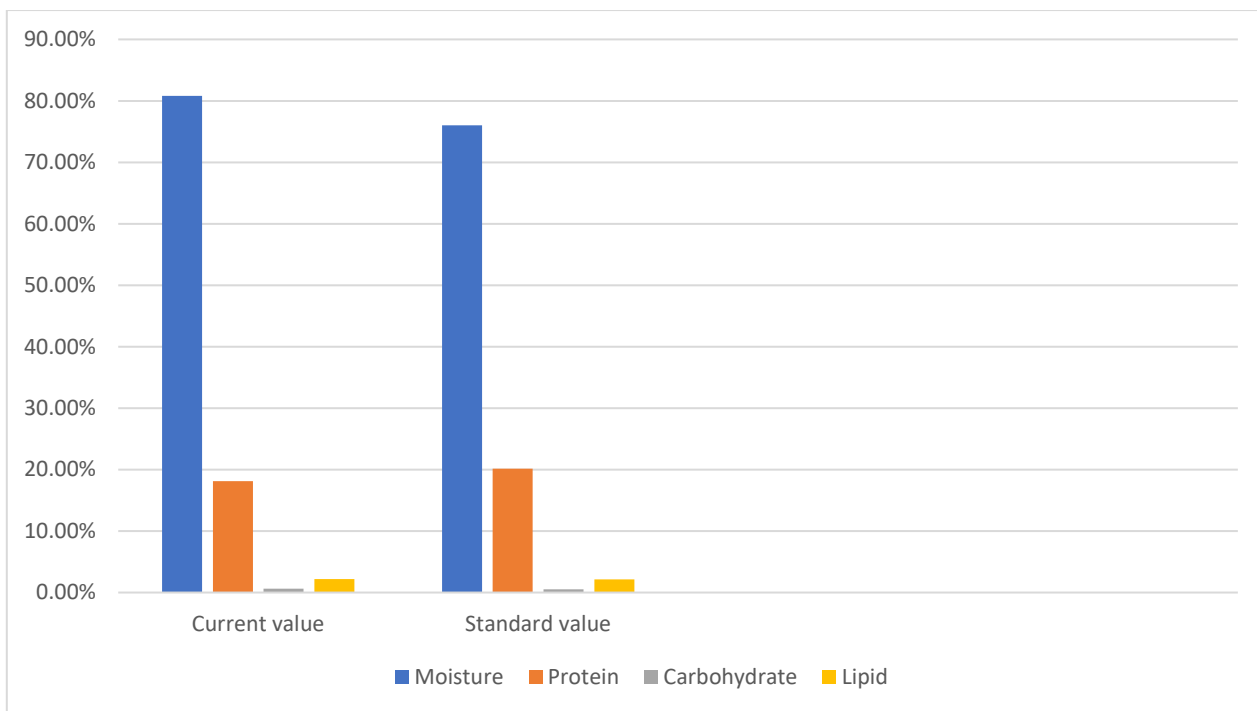
Using a Phenom Pro model desktop SEM, (Sathyabama University, Chennai). To acquire the most dehydrated powders, dried samples were conditioned for one week at room temperature in a desiccator with silica gel before being subjected to SEM analysis. Using a scanning electron microscope, the dehydrated samples were put on aluminum specimen holders, coated with palladium and gold, and analyzed.

CHAPTER 5

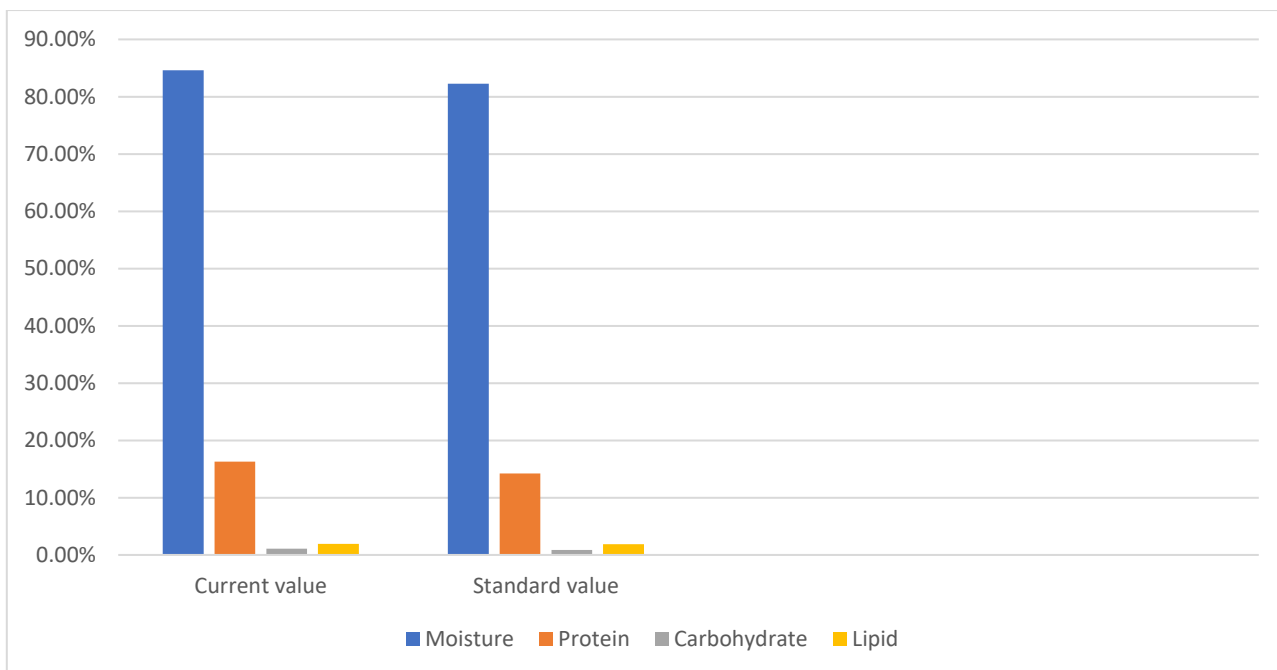
RESULTS AND DISCUSSION

5.1 PROXIMATE ANALYSIS

Tables 1 and 2 of the marine crab biochemical composition (%) revealed significant variations ($P < 0.05$) between the two species. *Portunus Sanguinolentus* (84.6%) and *Portunus Pelagicus* (80.8%) had the highest moisture content, respectively. Higher amount of protein present in *Portunus Pelagicus* (18.12%) and after that *Portunus Sanguinolentus* (16.3%). Then comparing the carbohydrate contents of the two species, *Portunus Sanguinolentus* had the greatest (1.12%) while *Portunus Pelagicus* had the lowest (0.65%). The lipid content of *Portunus Pelagicus* (1.92%) was much greater than that of *Portunus Sanguinolentus* (2.19%). When it comes to nutrition, biochemical research is crucial. Age, sex, season, and dietary habits of marine invertebrates all affect the biochemical makeup of their edible tissues. Seawater temperature and salinity (Oliveira *et al.*, 2007). Proteins are incredibly versatile molecules with a wide range of applications. Apart from acting as building blocks for all living things, they also have a variety of roles in metabolism control, transport, defense, and catalysis (Trudy and James, 2010). Lipids are more than twice as energy-dense as protein and carbohydrates, making them extremely effective energy sources. According to (Rosa and Nunes 2003) (Vinegre *et al.*, 2007), the proximate makeup of edible tissues typically reflects their physiological roles, metabolic demands, and available nutrition.



Graph 5.1.1: Biochemical value of Portunus Pelagicus (Blue Swimmer Crab)



Graph 5.1.2: Biochemical Value of *Portunus Sanguinolentus* (Three Spotted Crab)

5.2 CHITOSAN EXTRACTION

Deprotenization, Demineralization, Decolorization, and Deacetylation were the methods used to extract chitosan. The resulting chitin and chitosan's physicochemical properties are significantly influenced by each of these processes. However, this conventional extraction method also generates chemical waste, necessitating the neutralization and purification of wastewater (Ali et al., 2018; Küçükgülmez, 2018). In this study, chitosan was extracted from crab chitin using basic and acidic treatments. 9.7% of chitin and 26.4% of chitosan were yielded from 10g of *Portunus Pelagicus*. Using *Portunus Sanguinolentus*, 8.7% chitin and 24.7% chitosan were produced. Even while this procedure also involves chemical treatments, using crab shells as a raw material for chitosan extraction offers a more sustainable approach to chitin utilization.



Fig 5.2.1: Chitosan Yield of *Portunus pelagicus* and *Portunus sanguinolentus*

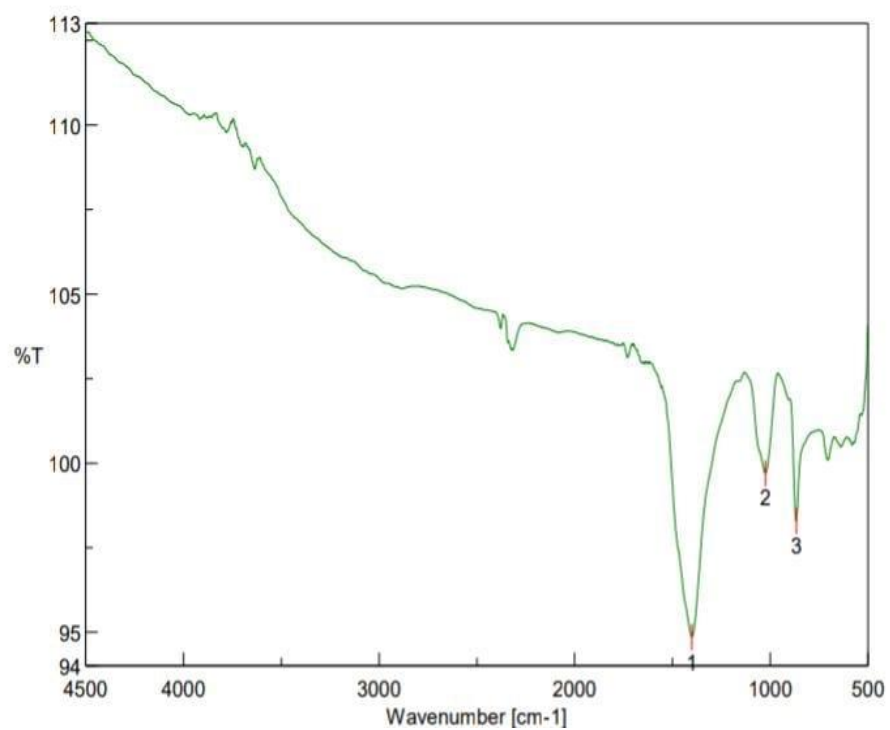


Fig 5.1: Utilizing the Three Spotted Crab (*P. Sanguinolentus*) and Blue Swimmer Crab (*P. pelagicus*) to extract chitin and chitosan

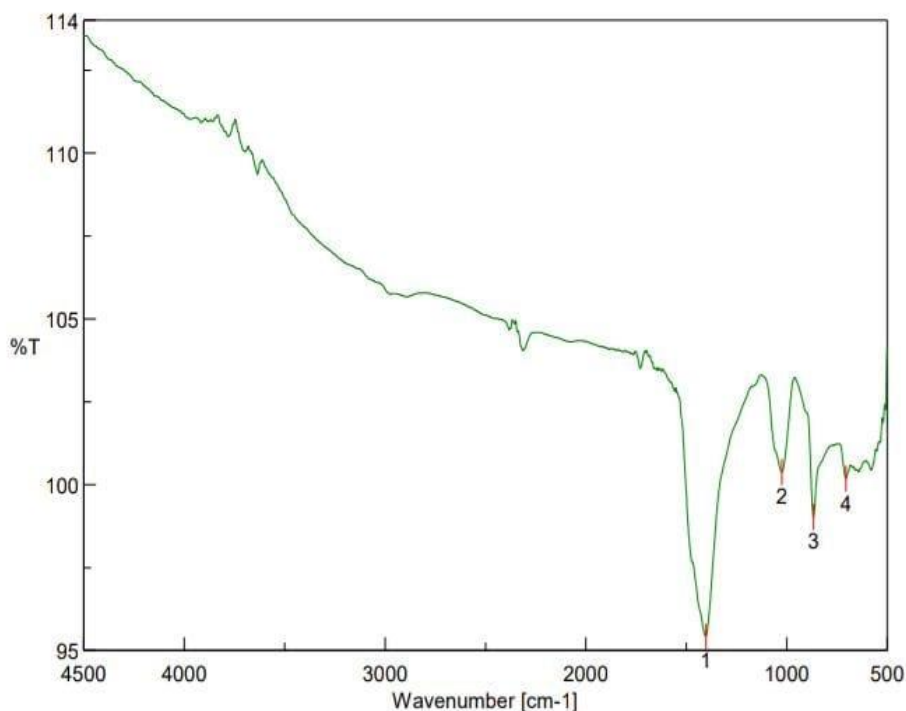
Table 5.2.1: yield of chitin and chitosan (%)

Species	Chitin (%)	Chitosan (%)
Portunus Pelagicus (Blue Swimmer Crab)	9.7%	26.4%
Portunus Sanguinolentus (Three Spotted Crab)	8.7%	24.7%

5.3. FTIR ANALYSIS



Graph 5.3.1: FTIR Result of P. Pelagicus



Graph 5.3.2: FTIR Result of *P. Sanguinolentus*

FTIR was used to analyze the chitosan's surface properties. Based on each functional group's unique wave number, the peak was assigned to them. Based on the FTIR Spectra analysis that was provided in (Graph:5.3.1)., the absorption peaks observed in the region of 1401.03, 1024.98 and 866.846 cm^{-1} for extracted crab chitosan of *Portunus pelagicus*. *Portunus Sanguinolentus* FTIR analysis presented in (Graph:5.3.2)., the location where the absorption peaks were seen 1402.96, 1024.98, 866.846, 705.819 cm^{-1} correspondingly, match the free amino groups' aliphatic, vibrating OH and NH stretching vibrations. Furthermore, the CO stretching from the -1acetyl group is represented by the peaks at 1647.21 and 1647.14 cm^{-1} , while the glucosamine stretching vibration is indicated by the peaks at 1024.43 and 1024.34 cm^{-1} . These findings align with earlier research (Si Trung and Bao, 2015). Additionally, according to Kumirska *et al.* (2010), An absorption peak corresponding to the ring stretching for β -1-4 glycosidic linkage falls between 892.82 and 892.5 cm^{-1} . The existence of these peaks in extracted crab chitosan suggests that the chemical structures of the two chitosan samples are identical. The similar peak frequencies found for extracted crab chitosan indicate that the chitosan's chemical structure was not

considerably changed by the extraction method employed in this investigation. It should be remembered, though, that the FTIR spectra may also be influenced by the chitosan's molecular weight and degree of deacetylation (Arantes *et al.*, 2015).

5.4 SOLUBILITY

The ability to dissolve A noteworthy distinction ($p < 0.05$) was seen in the solubility of the chitosan extracted from *Portunus Pelagicus* were (42.38%) and *Portunus Sanguinolentus* were (37.16%) and commercial chitosan (73.85%) respectively. The solubility of the both *Portunus Pelagicus* and *Portunus Sanguinolentus* chitosan extract was found to be lower than that of commercial chitosan, possibly as a result of insufficient protein removal. The presence of protein impurities in the sample during the analysis procedure could negatively affect the outcomes, since this method's chemical foundation depends on the reaction with the amino group (Fernandez-Kim 2004). Furthermore, numerous efforts have been undertaken to improve chitosan's water solubility. Although chitosan is hydrophilic by nature and possesses a polymer backbone made of hydrophilic functional groups, it is often insoluble in water and the majority of common organic solvents. Chitosan is insoluble in both aqueous and organic solvents due to its crystalline structure, which is the result of significant intramolecular and intermolecular hydrogen bonding between the chains and sheets, respectively.

5.5 SEM ANALYSIS

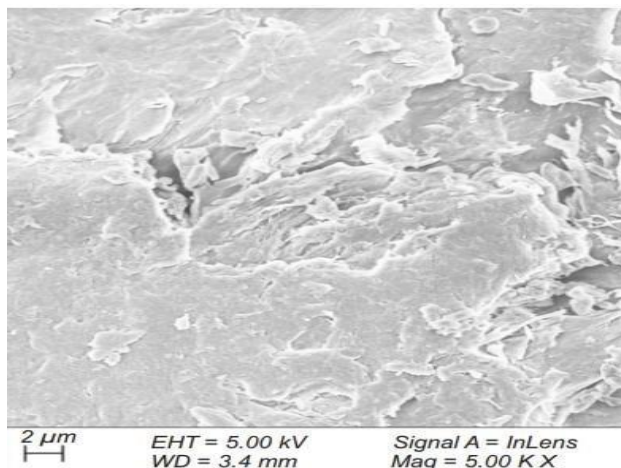


Fig 5.5.1: SEM image of *Portunus sanguinolentus* Crab

Chitosan

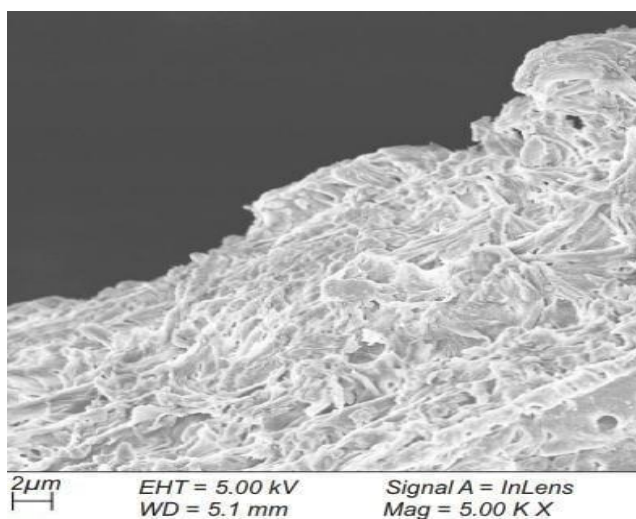


Fig 5.5.2: SEM image of *Portunus pelagicus* Crab Chitosan

The SEM pictures of commercial and crab chitosan were examined in the current investigation. It was demonstrated that following the N-deacetylation of crab chitosan, a distinctly ordered microfibrillar crystalline structure was produced. The reason for the microfibrillar crystalline structure observed in crab chitosan is thought to be the high degree of deacetylation (DD) of chitosan derived from *P. sanguinolentus* and *P. pelagicus* exoskeleton, which causes the production of crystalline areas in chitosan.

Microfibrillar structures are thought to be caused by the unique, layered, fibrous structure of chitin found in the exoskeleton of crustaceans.

5.6 ANTIMICROBIAL ACTIVITY



Fig 5.6.1: Zone of inhibition formation

The crab shell (*Portunus Pelagicus*) and (*Portunus Sanguinolentus*) chitosan gel's antibacterial activity displayed a maximal zone of inhibition of 0.9mm and 0.8mm against *Aeromonas hydrophilia*.

P. pelagicus showed more activity than *P. sanguinolentus*. In the present study, the moisture content of *Portunus Pelagicus* (80.8%) was higher than the other study in blue swimmer crab (76.0%). And that the Three spotted crab *Portunus Sanguinolentus* moisture level (84.6%) is higher than the other study of Three spotted crab (82.25) (Umer., et al 2021). In the present study says protein content of *Portunus Pelagicus* (18.12%) is lower than the other study of Blue swimmer crab (20.15%) (Ramamoorthy et., al 2016). In the current study says among various species *Portunus Sanguinolentus* carbohydrate value (1.12%) is higher than *Portunus Pelagicus* (0.65). (Umer et., al 2021) recorded the carbohydrate value of both *Portunus Pelagicus* and *Portunus Sanguinolentus* (0.54%) and (0.89%) respectively. Lipid content of both *Portunus Pelagicus* and *Portunus Sanguinolentus* were (2.19%) and (1.92%) respectively. Another study says *Portunus Pelagicus* has (2.15%) of lipid and *Portunus Sanguinolentus* were (1.86%).

CHAPTER 6

SUMMARY AND CONCLUSION

This Study clearly shows It may be determined that the proteins in the sea crabs from various species found around the Pudukkottai shore were of high grade. According to these indices, crabs are both very nutritious for human consumption and ideal for processing into a variety of crab products. Crabs like *P. pelagicus* and *P. sanguinolentus* are the most readily available dietary source of EPA and DHA. From this, it follows that the meat of the crab (*P. pelagicus* and *P. sanguinolentus*) is most suited as a protein and mineral supplement to maintain a balance in human diet. Therefore, eating crabs (*P. Pelagicus* and *P. sanguinolentus*) would be beneficial for human nutrition as a good source of nutrients. It is not advised to regularly eat fish since they contain hazardous levels of mercury, especially those found in higher-ranking fish. The results of the study show that chitosan obtained from the exoskeleton of *P. sanguinolentus* and *P. pelagicus* may be a more practical and affordable source of chitosan with superior physicochemical and biological properties than chitosan purchased from a shop. The results of this work may have implications for future investigations into the synthesis of new chemicals with antioxidant and antibacterial properties. This supports the claim that crab shells may be effectively used to produce chitosan, which is used in the culinary, pharmaceutical, and biomedical industries, among others. FTIR and SEM were used to analyze the resulting chitosan, and it was discovered to have antibacterial action against *Aeromonas hydrophilia*.

REFERENCES

- Agarwal, M., Nagar, D. P., Srivastava, N., & Agarwal, M. K. "Chitosan nanoparticles-based drug delivery: An update." *Int. J. Adv. Multidiscip. Res* 2.4, (2015): 1-13.
- Ahing, F. A., & Wid, N. Optimization of shrimp shell waste deacetylation for chitosan production. *International Journal of advanced and applied sciences*, 3(10), (2016): 31-36.
- An, H. K., B. Y. Park, and D. S. Kim. "Crab shell for the removal of heavy metals from aqueous solution." *Water research* 35.15 (2001): 3551-3556.
- Barber, Bruce. "Natural botanicals: Nature's pathway to better health and wellness." (2013): 7-59.
- Barrento, S., Marques, A., Teixeira, B., Vaz-Pires, P., & Nunes, M. L. Nutritional quality of the edible tissues of European lobster *Homarus gammarus* and American lobster *Homarus americanus*. *Journal of agricultural and food chemistry*, 57(9), (2009): 3645-3652.
- Barrento, S., Marques, A., Vaz-Pires, P., & Nunes, M. L. Cancer pagurus (Linnaeus, 1758) physiological responses to simulated live transport: Influence of temperature, air exposure and AQUIS®. *Journal of Thermal Biology*, 36(2), (2011): 128-137.
- Budge, S. M., Iverson, S. J., & Koopman, H. N. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. *Marine Mammal Science*, 22(4), (2006): 759-801
- Cacciari, M., Mangano, M. L., & Nason, P. Gluon PDF constraints from the ratio of forward heavy-quark production at the LHC at $\sqrt{s} = 7$ and 13 TeV. *The European Physical Journal C*, 75, (2015): 1-20.
- Chen, D. W., Zhang, M., & Shrestha, S. (2007). Compositional characteristics and nutritional quality of Chinese mitten crab (*Eriocheir sinensis*). *Food Chemistry*, 103(4), (2007): 1343-1349.
- Condrey, R. E., & Gelpi, C. G. Blue crab. *Callinectes sapidus*, (2010): 2009-043.

Davies, G. J., Knight, D. P., & Vollrath, F. Chitin in the silk gland ducts of the spider *Nephila edulis* and the silkworm *Bombyx mori*. *PloS one*, 8(8), (2013): e73225

Elegbede, I. O., & Fashina-Bombata, H. A. Proximate and mineral compositions of common crab species [*Callinectes pallidus* and *Cardisoma armatum*] of Badagry Creek, Nigeria. *Poultry, Fisheries and Wildlife Sciences*, 2(1), (2013): 110-115.

Hassan, H. U., Razzaq, W., & Masood, Z. Elemental composition of three-spot swimming crab *Portunus sanguinolentus* (Herbst, 1783) shell from the coasts of Sindh and Balochistan, Pakistan. *Environmental Science and Pollution Research*, 29(17), (2022): 25679-25684.

Heu, M. S., Kim, J. S., & Shahidi, F. (2003). Components and nutritional quality of shrimp processing by-products. *Food chemistry*, 82(2), (2003): 235-242.

Islam, M. B., Sarkar, M. M., Rahman, M. R., Mala Khan, M. K., Mirola Afroze, M. A., Hasan, M. A., ... & Sarkar, M. S. U. Fatty acid profile of freshwater crab (*Paratelphusa lamellifrons*) from Padma River of Rajshahi City, Bangladesh, (2017).

Kathirvel, M. Mud crab in hand book of aqua farming of shrimp lobster and mud crabs. *MPEDA Publication, Cochin*, 72, (1993)

Kaya, M., Dudakli, F., Asan-Ozusaglam, M., Cakmak, Y. S., Baran, T., Montes, A., & Erdogan, S. Porous and nanofiber α -chitosan obtained from blue crab (*Callinectes sapidus*) tested for antimicrobial and antioxidant activities. *LWT-Food Science and Technology*, 65, (2016):1109-1117.

Krisfalusi-Gannon, J., Waleed, A., Dellinger, K., Robertson, L., Brady, T. E., Goddard, M. K. M., ... & Dellinger, A. L. The role of horseshoe crabs in the biomedical industry and recent trends impacting species sustainability. *Front Mar Sci*, (2018).

Kunjachan, S., Jose, S., & Lammers, T. Understanding the mechanism of ionic gelation for synthesis of chitosan nanoparticles using qualitative techniques. *Asian Journal of Pharmaceutics (AJP)*, 4(2), (2010).

Lage Yusty, M. A., Vilasoa Martínez, M., Álvarez Pérez, S., & López Hernández, M. J. C. Chemical composition of snow crab shells (*Chionoecetes opilio*), (2011).

Laly, S. J., Anupama, T. K., Ashok Kumar, K., Sankar, T. V., & Ninan, G. Changes in biogenic amines, biochemical and microbial attributes of three spotted crab (*Portunus sanguinolentus*) during iced and refrigerated storage. *Journal of Food Science and Technology*, 58, (2021): 2197-2205.

Liu, D., Li, Z., Zhu, Y., Li, Z., & Kumar, R. Recycled chitosan nanofibril as an effective Cu (II), Pb (II) and Cd (II) ionic chelating agent: adsorption and desorption performance. *Carbohydrate polymers*, 111, (2014): 469-476.

Mahaffey, K. R. Fish and shellfish as dietary sources of methylmercury and the ω -3 fatty acids, eicosahexaenoic acid and docosahexaenoic acid: risks and benefits. *Environmental research*, 95(3), (2004):414-428.

Maulvault, A. L., Machado, R., Afonso, C., Lourenço, H. M., Nunes, M. L., Coelho, I., ... & Marques, A. (2011). Bioaccessibility of Hg, Cd and is in cooked black scabbard fish and edible crab. *Food and chemical toxicology*, 49(11), (2011): 2808-2815.

Nehring, S. Invasion history and success of the American blue crab *Callinectes sapidus* in European and adjacent waters. In *In the wrong place-alien marine crustaceans: distribution, biology and impacts* (pp. 607-624). Dordrecht: Springer Netherlands, (2011).

Omura, Y., Shigemoto, M., Akiyama, T., Saimoto, H., Shigemasa, Y., Nakamura, I., & Tsuchido, T. Antimicrobial activity of chitosan with different degrees of acetylation and molecular weights. *Biocontrol Science*, 8(1), (2003): 25-30.

Opeh, P. B., Udo, P. J., & Edem, E. T. Comparative studies on the food and mineral content of the shell of *egeria radiata* (bivalvia) and *crassostrea rhizophorea* (perecypode) of *lameillebrancha* of the Cross River, Nigeria. *International Journal of Current Research in Life Sciences*, 7(10), (2018): 2757-2760.

Paolisso, M. Taste the traditions: Crabs, crab cakes, and the Chesapeake Bay blue crab fishery. *American Anthropologist*, 109(4), (2007): 654-665

Radhakrishnan, E. V., Manisseri, M. K., & Nandakumar, G. Status of research on crustacean resources, (2007).

Rangasamy, E., LBS, V. V., Kamali, M. D., & Muniyandi, M. Determination of proximate composition on some edible crabs with special reference to nutritional aspects collected from coastal waters of Rameshwaram, Tamil Nadu. *Food Chemistry Advances*, (2024):100686.

Rosa, R., & Nunes, M. L. Tissue biochemical composition in relation to the reproductive cycle of deep-sea decapod *Aristeus antennatus* in the Portuguese south coast. *Journal of the Marine Biological Association of the United Kingdom*, 83(5), (2003): 963-970.

Samuel, N. J., & Soundarapandian, P. Fishery Potential of Commercially Important Crab *Portuns sanguinolentus* (Herbst) along Parangipettai Coast, South East Cost of India. *Int. J. Anim. Veter. Adv*, 1(2), (2009): 99-104.

Serhan, C. N., Chiang, N., & Van Dyke, T. E. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nature Reviews Immunology*, 8(5), (2008): 349-361.

Skonberg, D. I., & Perkins, B. L. Nutrient composition of green crab (*Carcinus maenus*) leg meat and claw meat. *Food chemistry*, 77(4), (2002): 401-404.

Soundarapandian, P., Ilavarasan, N., Varadharajan, D., & Gangatharan, K. Seed production of commercially important portunid crab, *Charybdis feriata* (Linnaeus). *Journal of Marine Science Research Development*, 3(2), (2013): 6.

Thorarinsdottir, K. A., Arason, S., Geirsdottir, M., Bogason, S. G., & Kristbergsson, K. Changes in myofibrillar proteins during processing of salted cod (*Gadus morhua*) as determined by electrophoresis and differential scanning calorimetry. *Food chemistry*, 77(3), (2002): 377-385.

US Department of Agriculture, Agricultural Research Service. USDA national nutrient database for standard reference, (2015).

Vikele, L., Laka, M., Sable, I., Rozenberga, L., Grinfelds, U., Zoldners, J., ... & Mauret, E. Effect of chitosan on properties of paper for packaging. *Cellul. Chem. Technol*, 51(1-2), (2017): 67-73.

Vilasoa-Martínez, M., Lopez-Hernandez, J., & Lage-Yusty, M. A. (2007). Protein and amino acid contents in the crab, *Chionoecetes opilio*. *Food chemistry*, 103(4), (2007):1330-1336.

Waugh, D. A., & Feldmann, R. M. Cuticle microstructure as a new tool in systematic paleontology. *Contributions to Zoology*, 72(2-3), (2003): 191-193.

Wu, H. J., Sun, L. B., Li, C. B., Li, Z. Z., Zhang, Z., Wen, X. B., ... & Li, S. K. Enhancement of the immune response and protection against *Vibrio parahaemolyticus* by indigenous probiotic *Bacillus* strains in mud crab (*Scylla paramamosain*). *Fish & shellfish immunology*, 41(2), (2014): 156-162.

Zafar, M., & Ahsan, M. N. Marketing and value chain analysis of mud crab (*Scylla* sp.) in the coastal communities of Bangladesh. In *value chain analysis and market assessment of coastal and marine aquatic products of Bangladesh*. *Bangladesh Fisheries Research Forum, Dhaka*, (2006): (pp. 25-53).