ISSN 2250-3153

# Extraction and Characterization of Water Soluble Chitosan from Parapeneopsis Stylifera Shrimp Shell Waste and Its Antibacterial Activity

**K. Kamala[*], P. Sivaperumal[**], R. Rajaram[***]**

[*] Ph.D., Scholar, CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai, Tamil Nadu.
[**] Junior Research Fellow, FRM Division, CIFE, Mumbai.
[***] Assistant Professor, Department of Marine Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu..

***Abstract-*** Preparation and characterization of water soluble chitosan was examined for their antibacterial activity from P. stylifera. The yield of crude chitosan and water soluble chitosan was 54.3 % and 87.8%. The FT-IR spectrum of chitin, chitosan and water soluble chitosan also determined and characterization was done and compared with standards. Compare to other bacterial strains S.auerus (18.3mm) having more potential antibacterial activity in crude chitosan as well as water soluble chitosan. Both chitosans might have the antibacterial activity which would be used in novel drugs from the shrimp shell waste.

***Index Terms***- Shrimp shell waste, Water soluble chitosan, FT-IR and Antibacterial

## I. INTRODUCTION

Chitin is a natural polysaccharide synthesized by a great number of living organisms and functions as a structural polysaccharide[1].Chitosan is the only pseudonatural cationic polymer which has many potential biomedical and other applications. Chitosan has been proved usefully for would dressing and bone tissue engineering[2-3]. It shows good performance in drug delivery and analgesia[4]. Chitosan has some beneficial properties, such as antimicrobial activity, excellent biocompatibility and low toxicity that promote its applications in many fields including food industry and pharmaceutics[5-7].

Chitosan is natural, non toxic, copolymer of glucosamine and N-acetylglucosamine prepared from chitin by deacetylation, which in turn, is a major component of the shells of crustaceans. It is found commercially in the waste products of the marine food processing industry[8-9]. Various chemical modifications have been investigated to try and improve chitosan's solubility and thus to increase its range of applications[10-11]. Recent studies on chitosan depolymerisation have drawn considerable attention, as the products obtained are more water-soluble. Beneficial properties of chitosan and its oligosaccharides include: antitumour[12]; neuroprotective[13]; antifungal and antibacterial[14-15]; and anti-inflammatory[16].

The antimicrobial activity of chitin, chitosan and their derivatives against different groups of microorganisms, such as bacteria, yeast, and fungi, has received considerable attention in recent years[17-18]. Two main mechanisms have been suggested as the cause of the inhibition of microbial cells by chitosan. One means is that the polycationic nature of chitosan interferes with bacterial metabolism by electrostatic stacking at the cell surface of bacteria[19-20]. The other is blocking of transcription of RNA from DNA by adsorption of penetrated chitosan to DNA molecules. In this mechanism the molecular weight of chitosan must be less than some critical value in order to be able to permeate into cell[21]. The antimicrobial activities of chitosan are greatly dependent on its physical characteristics, most notably molecular weight (Mv) and degree of deacetylation (DD). Chitosan with a higher degree of deacetylation tends to have a higher antimicrobial activity[22]. Chitosan is more effective than chito-oligosaccharides (COS) in inhibiting growth of bacteria; for example, water insoluble chitosans exhibited higher antimicrobial effect against E. coli than COS[23]. The preparation and characterization of chitosan and its biomedical applications are still limited. In this study, the antibacterial activities of water soluble chitosan against urinary tract infection bacterial suspension (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella oxytoca, Staphylococcus aureus, Streptococcus pnemoniae, Klebsiella Pneumoniae, Salmonella typhi* were compared to chitosan prepared from shrimp shell waste (*Parapeneopsis stylifera*). Hydrogen peroxide was used to degrade the chitosan into water-soluble chitosan. The long term aim of this work is to increase the novel drug application from chitosan and water soluble chitosan in the medical industry.

## II. MATERIALS AND METHODS

### 2.1. Chemicals

Hydrogen peroxide, acetic acid, hydro chloric acid and sodium hydroxide and all the other chemicals and reagents are purchased from Sigma Chemical Co.

### 2.2. Extraction of chitin from shrimp shells:

The *P. stylifera* shrimp shell wastes were collected from the Versova landing centre, Mumbai. Shells are removed and thoroughly washed with running tap water with sample care so as to remove sand adhered to it, the exoskeleton were subjected to shade drying for 2 days and then placed in hot air oven for at $60^0$C for 24 hours. The preparation of chitin from shrimp shell followed by[24] with some modification. Diluted HCl solution was used for demineralization. One hundred grams of shrimp shell powder was immersed in 1000 ml of 7% (w/w) HCl at room temperature (25°C) for 24 h. After filtration with mid speed filter paper, the residue was washed with distilled water to neutral.

Then the residue was immersed in 1000 ml of 10% (w/w) NaOH at 60°C for 24 h for deproteination. The proteins were removed by filtration. Distilled water was used to wash the residue to neutral. Then the shrimp shell residue was subjected to the above program for two times. 250 ml of 95% and absolute ethanol were sequentially used to remove ethanol-soluble substances from the obtained crude chitin and to dehydrate. An air oven was taken to dry the chitin at 50°C overnight.

**2.3. Preparation of chitosan and water soluble chitosan:**

The preparation of chitosan and water soluble chitosan followed by[24] with some modification. The chitin (10g) was put into 50% NaOH at 60°C for 8h to prepare crude chitosan. After filtration, the residue was washed with hot distilled water at 60°C for three times. The crude chitosan (4.1g) was obtained by drying in an air oven at 50°C overnight. One gram of crude chitosan was added into 20 ml of 2% (w/w) acetic acid in a water-bath shaker. The conditions were set as follows: $H_2O_2$ level (4%), time (4 h) and temperature (60°C). After reaction, 10% NaOH was used to adjust the solution to neutrality. The residue was removed by filtration, while twofold volumes of ethanol were added to the filtrate. The crystal of water-soluble chitosan was liberated after incubation at ambient condition overnight and dried in an air oven at 50°C. The recovery (%) was calculated as (the weight of water-soluble chitosan/the weight of crude chitosan) ×100.

**2.4. Fourier Transform - Infra Red spectroscopy (FT-IR):**

The chitin, chitosan, water soluble chitosan, standard chitin and chitosan were determined using FT-IR spectrometer (Bio-Rad FTIS-40 model, USA). Sample (10 µg) was mixed with 100 µg of dried Potassium Bromide (KBr) and compressed to prepare a salt (10 mm diameter).

**2.5. Assay of antibacterial activity of crude and water-soluble chitosan:**

This assay was done according to the method of[25] with some modifications. 50 µl of urinary tract infection bacterial suspension (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella oxytoca, Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae*, and *Salmonella typhi)* was inoculated in a petri dish with Muller Hinton agar medium. After incubation at 37°C for 24h, the diameters of inhibition zones (in mm) were measured. Sterilized distilled water was used for control. All the Pathogenic bacterial strains were obtained from Raja Muthiah Medical College, Annamalai University. The concentrations of crude chitosan and water-soluble chitosan used in this assay were 500µg and 1mg respectively. The positive control was used as streptomycin and negative control was sterile double distilled water.

## III. RESULTS

The yield of chitin and chitosan from *P. stylifera* shrimp shell waste was 32% and 54.31%, respectively. Chitin was prepared by using acid and alkaline treatments; the yield of chitin was 32% in the total weight of the dried *P. stylifera* shells, after N- acetylation, the yield of chitosans were in the range of 54.31%. Whereas in the case of water soluble chitosan obtained from the chitosan of *P. stylifera* was 87.8%.

Infrared spectroscopy of the structure changes of initial chitin, chitosan and water soluble chitosan were confirmed by FTIR spectroscopy with standard chitin and chitosan (Fig: 1-5). The FT-IR spectrum of chitin revealed that the peak 3293 $cm^{-1}$ indicates the presence of OH stretching coupled and 2961 $cm^{-1}$ indicates the presence of NH stretching. Compare to standard chitin this stretching wave number was more or less same. The wave number 2933 $cm^{-1}$ characteristic of asymmetrical stretching of $CH_2$, whereas 1214 $cm^{-1}$, 1138 $cm^{-1}$, 933 $cm^{-1}$ and 743 $cm^{-1}$ positions of the spectrums are the characteristic C=O stretching, $CN_3H_5^+$, COH, CH, C-O and Skeletal stretch respectively (Table-1). These asymmetrical stretching, bending and skeletal stretch indicated that the presence of the chitin.

The standard chitosan peaks, six were found to be prominent and were representing chitosan (Structural unit - 3436$cm^{-1}$, (-NH2) Amide II 1636$cm^{-1}$, PO3 $4^-$ 1322$cm^{-1}$, (NH) Amide III 894$cm^{-1}$ and NH-out of plane bending 778$cm^{-1}$. The peak of crude chitosan and water soluble chitosan peak stretching was near by the standard chitosan wave number absorption only. This wave number absorption implies the substantiation of the chitosan and water soluble chitosan from the *P. stylifera* shrimp shell waste (Table 2).

*In-vitro* antibacterial screening of chitosan and water soluble chitosan from *P. stylifera* against selected clinical isolates were performed and zone of inhibition were given in Table 3. The concentration of chitosan and water soluble chitosan were 500µg and 1mg/ml respectively. All the experiment was done as a triplicate. The maximum inhibition zone (18.3 mm) was observed against the *S. aureus* in water soluble chitosan (1mg/ml). Compare to positive control streptomycin (11.6 mm), water soluble chitosan zone of inhibition was high. The range of inhibition in crude chitosan 1.4 mm to 8.9 mm. highest zone of inhibition was observed in *S.aureus* followed by *E.coli*, and *P.aeuroginosa*. The water soluble chitosan zone of inhibition range was high compare to crude chitosan as well as concentration wise also higher activity observed from the water soluble chitosan. Both crude and water-soluble chitosan showed higher inhibition activity against *S. aureus*, compared with the other bacteria tested. This indicated that both chitosans might have the antibacterial inhibition mechanism.

## IV. DISCUSSION

The yield of chitin was 32% in the total weight of the dried *P. stylifera* shells, after N- acetylation, the yield of chitosans were in the range of 54.31%.[26] reported that, the crude polysaccharide was obtained as a water soluble dust-coloured powder from plant root of *B. chinense* by hot water extraction. The total yield of crude water-soluble polysaccharides was 6.5% of the dried material. The cuttlebone of *Sanguisorba officinalis* was found to be 20% of chitin[27-28], whereas in general, the squid/ cuttlefish reported 3-20% of chitin[29]. One of the major problems related to the preparation of pure chitins is keeping a structure as close as possible than the native form is to minimize the partial deacetylation and chain degradation caused by demineralization and deproteinization applied during process of the raw materials. Shrimp chitin showed no color and odor. Chitin occurs naturally partially deacetylated (with a low content of glucosamine units),

depending on the source[30]; nevertheless, both α - and β - forms are insoluble in all the usual solvent, despite natural variation in crystallinity. The insolubility is a major problem that confronts the development mechanisms and uses of chitin. But present study in the case of water soluble chitosan we obtained 87.8%. The β- chitin is more reactive than the α- form, an important property with regard to enzymatic and chemical transformations of chitin[31].

[32]observed that IR spectrum of chitosan oligomers showed peaks assigned to the polysaccharide structure at 1155, 1078, 1032, and 899 cm−1, and a strong amino characteristic peak at around 3425, 1651, and 1321 cm−1 were assigned to amide I and III bands, respectively. The peak at 1418 cm−1 is the joint contribution of bend vibration of OH and CH. [33] reported that IR spectrum of water soluble polysaccharide from *Bupleurum chinense* revealed a typical major broad stretching peak at 3411 $cm^{-1}$ for the hydroxyl group, and a weak band at 2919 $cm^{-1}$ showed C–H stretching vibration. The broad band at 1610 $cm^{-1}$ was due to the bound water. The band at 842 $cm^{-1}$ and 877 $cm^{-1}$ indicated a- and b-configurations of the sugar units simultaneously existing in the polysaccharide. In the present study crude chitosan and water soluble chitosan observation band also similar to the following wave number such as chitosan 3429 $cm^{-1}$, 1568 $cm^{-1}$,1559 $cm^{-1}$, 1405 $cm^{-1}$, 1105 $cm^{-1}$ and 929 $cm^{-1}$. The water soluble chitosan stretching peak at 3399 $cm^{-1}$ and 1654 $cm^{-1}$,1647 $cm^{-1}$, 1078 $cm^{-1}$ and 644 $cm^{-1}$.

The antimicrobial activity of chitin, chitosan, and their derivatives against different groups of microorganisms, such as bacteria, yeast, and fungi, has received considerable attention in recent years. Two main mechanisms have been suggested as the cause of the inhibition of microbial cells by chitosan. The interaction with anionic groups on the cell surface, due to its polycationic nature, causes the formation of an impermeable layer around the cell, which prevents the transport of essential solutes. It has been demonstrated by electron microscopy that the site of action is the outer membrane of gram negative bacteria. The permeabilizing effect has been observed at slightly acidic a condition in which chitosan is protonated, but this permeabilizing effect of chitosan is reversible[34].

Chitosan has been confirmed to possess a broad spectrum of antimicrobial activities[35]. However, the low solubility of chitosan at neutral pH limits its application. In this study $H_2O_2$ was taken to degrade the chitosan into water soluble chitosan. Several studies prove that an increase in the positive charge of chitosan makes it bind to bacterial cell walls more strongly[36-37]. [38]have mentioned that molecular weight is the main factor affecting the antibacterial activity of chitosan, from the results obtained. In contrast, some authors have not found a clear relationship between the degree of deacetylation and antimicrobial activity. These authors suggest that the antimicrobial activity of chitosan is dependent on both the chitosan and the microorganism used[39-40]. [41]studied the antimicrobial activity of hetero-chitosans with different degrees of deacetylation and Molecular weight against three Gram negative bacteria and five Gram-positive bacteria and found that the 75% deacetylated chitosan showed more effective antimicrobial activity compared with that of 90% and 50% deacetylated chitosan. In the present study 87.8% deacetlated water soluble chitosan showed higher antibacterial activity against *S.auerus* than crude chitosan. This indicated that both chitosans might have the antibacterial activity which could be used in pharmacological research.

## V. CONCLUSION

We deduce that, the continuing and overwhelming contribution of water soluble chitosan to the development of new pharmaceuticals are clearly evident and need to be explored. After taking in to consideration the immense side effects of synthetic drugs, great attention has to be paid for the discovery of novel drugs from marine crustaceans waste.

## ACKNOWLEDGEMENT

Authors are highly thankful to HOD, Fisheries Resource Management, CIFE, Mumbai and The Director, CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University for providing facilities.

## REFERENCES

[1] Abdou, E. S., Nagy, K. S. A., & Elsabee, M.Z, Extraction and characterization of chitin and chitosan from local sources. Bioresource Technology, 2008: 99 1359−1367.

[2] Felt, O., Buri, P., & Gurny, R. Chitosan: A unique polysaccharide for drug delivery. Drug Development and Industrial Pharmacy, 1998: 24, 979−993.

[3] Li, Z., Ramay, H. R., Hauch, K. D., Xiao, D., & Zhang, M. Chitosan-alginate hybrid scaffolds for bone tissue engineering. Biomaterials, 2005: 26, 3919−3928.

[4] Wang, L., Khor, E., Wee, A., & Lim, L. Y. Chitosan–alginate PEC membrane as a wound dressing: Assessment of incisional wound healing. Journal of Biomedical Materials Research, 2002: 63, 610−618.

[5] Okamoto, Y., Kawakami, K., Miyatake, K., Morimoto, M., Shigemasa, Y., & Minami, S. Analgesic effects of chitin and chitosan. Carbohydrate Polymers, 2002: 49, 249−252.

[6] Muzzarelli RAA, Muzzarelli C. Chitosan chemistry: Relevance to the biomedical sciences. Adv Polymer Sci., 2005:186: 151-209.

[7] Ouattara B, Simard RE, Piette G, Begin A, Holley RA. Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with chitosan. Int J Food Microbiol., 2000:62: 139-148.

[8] Tokura S, Tamura H. Chitin and chitosan. In: Kamerling JP. (ed). Comprehensive glycoscience from chemistry to systems biology, vol. 2. Oxford: Elsevier; 2007: p. 449-474.

[9] Khanafari, A., Marandi, R., and Sanatei, Sh. Recovery of chitin and chitosan from shrimp waste by chemical and microbial methods. Iranian Journal of Environmental Health Science and Engineering, 2008: 5(1), 19-24.

[10] Limam, Z., Selmi, S., Sadok, S., and El-abed, A. Extraction and characterization of chitin and chitosan from crustacean by-products: biological and physicochemical properties. African Journal of Biotechnology, 2011: 10(4), 640-647.

[11] Park, B. K., and Kim, M. M. Applications of chitin and its derivatives in biological medicine. International Journal of Molecular Science, 2010: 11, 5152-5164.

[12] Zhang, J., Xia, W., Liu, P., Cheng, Q., Tahirou, T., and Li, B. Chitosan modification and pharmaceutical/biomedical application. Marine Drugs, 2010: 8, 1962-1987.

[13] Quan, H., Zhu, F., Han, X., Xu, Z., Zhao, Y., and Miao, Z. Mechanism of antiangiogenic activities of chitooligosaccharides may be through inhibiting heparanase activity. Medical Hypotheses, 2009: 73, 205-206.

[14] Pangestuti, R., and Kim, S. K. Neuroprotective properties of chitosan and its derivatives. Marine Drugs, 2010: 8, 2117-2128.

[15] Fernandes, J. C., Tavaria, F. K., Soares, J. C., Ramos, O. S., Monteiro, M. J. & Pintado, M. E., Antimicrobial effects of chitosans and chitooligosaccharides, upon Staphylococcus aureus and Escherichia coli, in food model systems. Food Microbiology, 2008: 25, 922-928.

[16] Wang, Y., Zhou, P., Yu, J., Pan, X., Wang, P., Lan, W. Antimicrobial effect of chitooligosaccharides produced by chitosanase from Pseudomonas CUY8. Asia Pacific Journal of Clinical Nutrition, 2007: 16, 174-177.

[17] Yang, E. J., Kim, J. G., Kim, J. Y., Kim, S., & Lee, N. Anti-inflammatory effect of chitosan oligosaccharides in RAW 264.7 cells. Central European Journal of Biology, 2010: 5, 95-102.

[18] Khanafari, A., Marandi, R., & Sanatei, Sh. Recovery of chitin and chitosan from shrimp waste by chemical and microbial methods. Iranian Journal of Environmental Health Science and Engineering, 2008: 5(1), 19-24

[19] Limam, Z., Selmi, S., Sadok, S., & El-abed, A. Extraction and characterization of chitin and chitosan from crustacean by-products: biological and physicochemical properties. African Journal of Biotechnology, 2011: 10(4), 640-647

[20] Chung, Y., Su, Y., Chen, C., Jia, G.,Wang, H., and Wu, J., Relationship between antibacterial activity of chitosan and surface characteristics of cell wall. Acta Pharmacologica Sin, 2004: 25, 932-936.

[21] Je, J., & Kim, S. Chitosan derivatives killed bacteria by disrupting the outer and inner membrane. Journal of Agricultural Food Chemistry, 2006: 54, 6629-6633.

[22] Liu, X., Yun, L., Dong, Z., Zhi, L., & Kang, D. Antibacterial action of chitosan and carboxymethylated chitosan. Journal of Applied Polymers Science, 2001: 79(7), 1324-1335.

[23] Acharya, B., Kumar, V., Varadaraj, M. C., Lalitha, R., & Rudrapatnam, N. Characterization of chito-oligosaccharides prepared by chitosanolysis with the aid of papain and pronase, and their bactericidal action against Bacillus cereus and E. coli. Biochemical Journal, 2005: 391, 167-175.

[24] Qin, C., Li, H., Xiao, Q., Liu, Y., Zhu, J., & Du, Y. Water-solubility of chitosan and its antimicrobial activity. Carbohydrate Polymers, 2006: 63, 367-374.

[25] Du, Y., Zhao, Y., Dai, S. & Yang, B. Preparation of water-soluble chitosan from shrimp shell and its antibacterial activity, Innovative Food Science and Emerging Technologies, 2009: 10, 103–107.

[26] Wang, H. Zhao, Y., Yang, M. M., Jiang, B. Y. M. & Rao, G. H. Identification of polyphenols in tobacco leaf and their antioxidant and antimicrobial activities. Food Chemistry, 2008: 107, 1399−1406.

[27] Sun, L., Feng, K., Jiang, R, Chen, J., Zhao Y., Ma, R. & Tong, H. Water-soluble polysaccharide from Bupleurum chinense DC: Isolation, structural features and antioxidant activity, Carbohydrate Polymers, (2010: 79, 180–183.

[28] Tolaimate A., Debrieres J., Rhazi M., Alagui A., Vincendon M. & Vottero P. On the influence of deacetylation process on the physicochemical characteristics of chitosan from squid chitin, Polymer, 2000: 41: 2463 - 2469.

[29] Tolaimate A., Debrieres J., Rhazi M. & Alagui A. Contribution to the preparation of chitin and chitosan with controlled physicochemical properties. Polymer, 2003: 44: 7939-7952.

[30] Patil YT. & Satam SB. Chitin and chitosan, treasure from crustacean shell waste. Sea Food Export J 2002: XXXIII(7): 31-38.

[31] Mathur NK. & Narang CK. Chitin and chitosan, versatile polysaccharides from marine animals. J Chem Edu., 1990: 67: 938- 942.

[32] Kurita K., Tomita K., Ishi S., Nishimura SI. & Shimoda K. β –Chitin as a convenient starting material for acetolysis for efficient preparation of N - cetylchitooligosaccharides. J Poly Sci A Poly Chem., 1993: 31: 2393 - 2395.

[33] Sun T., Zhou D., Xie, J. Mao, F. Preparation of chitosan oligomers and their antioxidant activity, Eur Food Res Technol., 2007: 225:451–456.

[34] Helander I, Nurmiaho-Lassila E, Ahvenainen R, Rhoades J, Roller S. Chitosan disrupts the barrier properties of the outer membrane of Gram-negative bacteria. Int J Food Microbiol., 2001: 71: 235-244.

[35] Chung, Y., Su, Y., Chen, C., Jia, G.,Wang, H.,Wu, J., Relationship between antibacterial activity of chitosan and surface characteristics of cell wall. Acta Pharmacologica Sin., 2004: 25, 932-936.

[36] Gerasimenko DV, Avdienko ID, Bannikova GE, Zueva OY, Varlamov VP. Antibacterial Effects of Water-Soluble Low-Molecular- Weight Chitosans on Different Microorganisms. Appl Biochem Microbiol., 2004:40(3): 253-257.

[37] Liu, N., Chen, X.-G., Park, H.-J., Liu, C.-G., Meng, X.-H., & Yu, L.J. Effect of Mv and concentration of chitosan on antibacterial activity of Escherichia coli. Carbohydrate Polymers, 2006: 64, 60-65.

[38] Chien P, Chou C. Antifungal activity of chitosan and its application to control post-harvest quality and fungal rotting of Tankan citrus fruit (Citrus tankan Hayata). J Sci Food Agric., 2006: 86: 1964-1969.

[39] Oh H, Kim Y, Chang E, Kim J. Antimicrobial Characteristics of Chitosans against Food Spoilage Microorganisms in Liquid Media and Mayonnaise. Biosci Biotechnol Biochem., 2001; 65(11): 2378- 2383.

[40] Park, B. K., & Kim, M. M. Applications of chitin and its derivatives in biological medicine. International Journal of Molecular Science, 2010: 11, 5152-5164.

[41] Park PJ, Je JY, Byun HG, Moon SH Kim SK. Antimicrobial Activity of Hetero-Chitosans and Their Oligosaccharides with Different Molecular Weights. J Microbiol Biotechnol., 2004: 14(2): 317-323.

## AUTHORS

**First Author** – K. Kamala, Ph.D., Scholar, CAS in Marine Biology, Annamalai University, Parangipettai-, Tamil Nadu. Email-kamal.actino@gmail.com
**Second Author** – P. Sivaperumal, Junior Research Fellow, Fisheries Resource management, CIFE, Mumbai-400061. Email-marinesiva86@gmail.com
**Third Author** – Dr. R. Rajaram, Assistan Professor, Department of Marine Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu. Email-dnabarcodingram@gmail.com

**Correspondence Author** – P. Sivaperumal, Junior Research Fellow, Fisheries Resource management, CIFE, Mumbai-400061. Email-marinesiva86@gmail.com

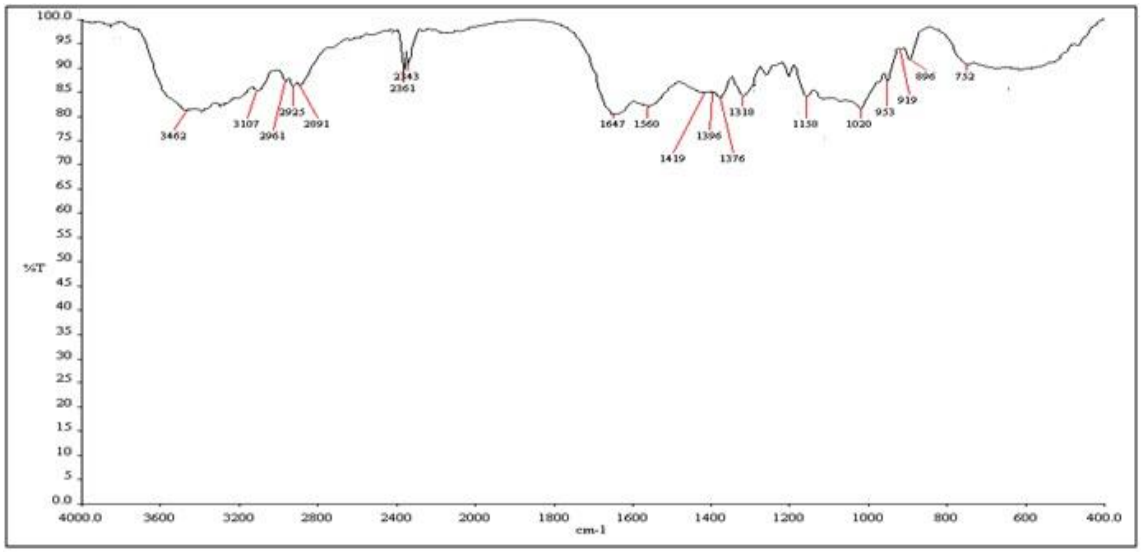


**Fig: 1 FT-IR spectrum of standard chitin**

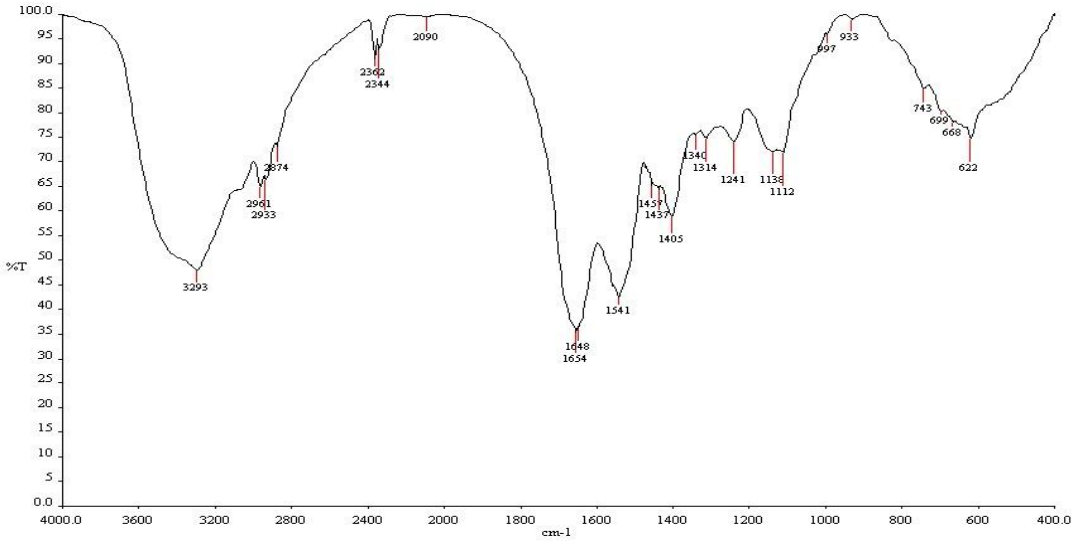


**Fig: 2 FT-IR spectrum of chitin from *P. stylifera* shrimp shell waste**

**Table-1: Main bands observed in the FT IR spectra of standard chitin and *P. stylifera* shrimp shell waste**

| **Vibration mode (Pearson et al., 1960)** | **Std. Chitin (α-chitin) ($cm^{-1}$)** | **Chitin from *P. stylifera* ($cm^{-1}$)** |
|---|---|---|
| OH stretching | 3462 | 3293 |
| NH stretching | 3107 | 2961 |
| Symmetric CH3 stretching and asymmetric CH2 stretching | 2925 | 2933 |
| Amide I band | 1647 | 1654 and1648 |
| Amide II band | 1560 | 1541 |

| | | |
|---|---|---|
| CH2 bending and CH3 deformation | 1419 | 1437 and 1405 |
| Amide III band and CH2 wagging | 1318 | 1314 |
| Asymmetric bridge O2 stretching | 1150 | 1214 |
| CO-stretching | 1020 | 1138 |
| CH3 wagging alone chain | 953 | 933 |
| NH-out of plane bending | 752 | 743 |

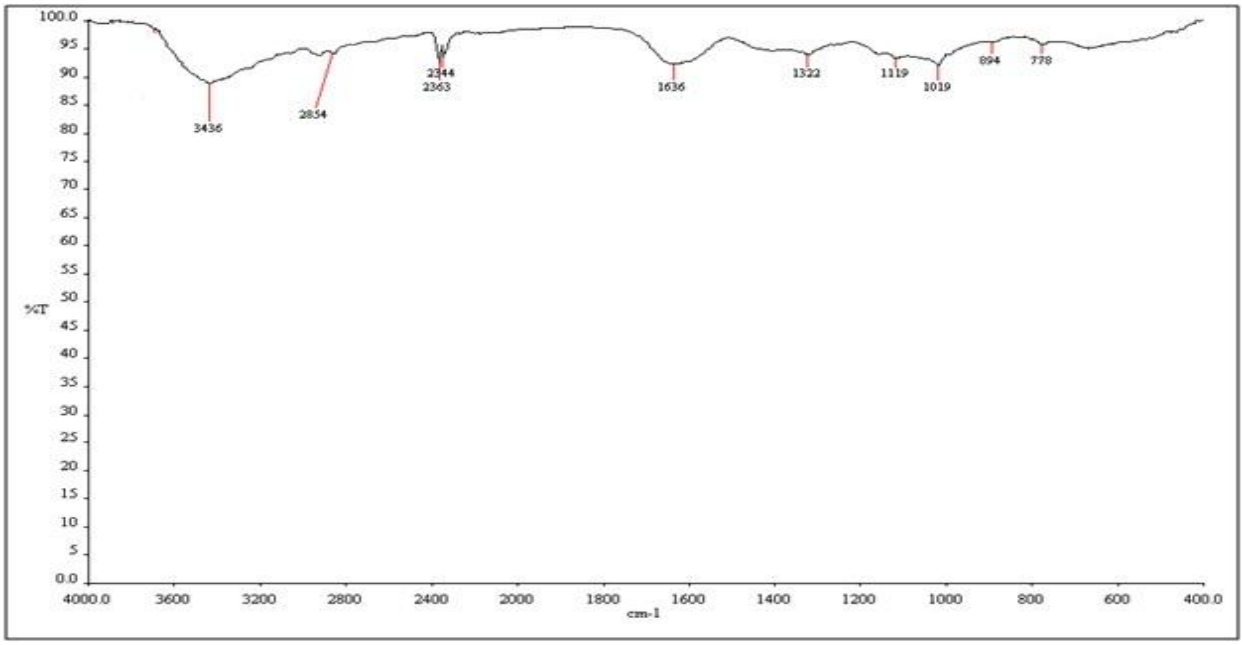


**Fig: 3 FT-IR spectrum of standard chitosan**

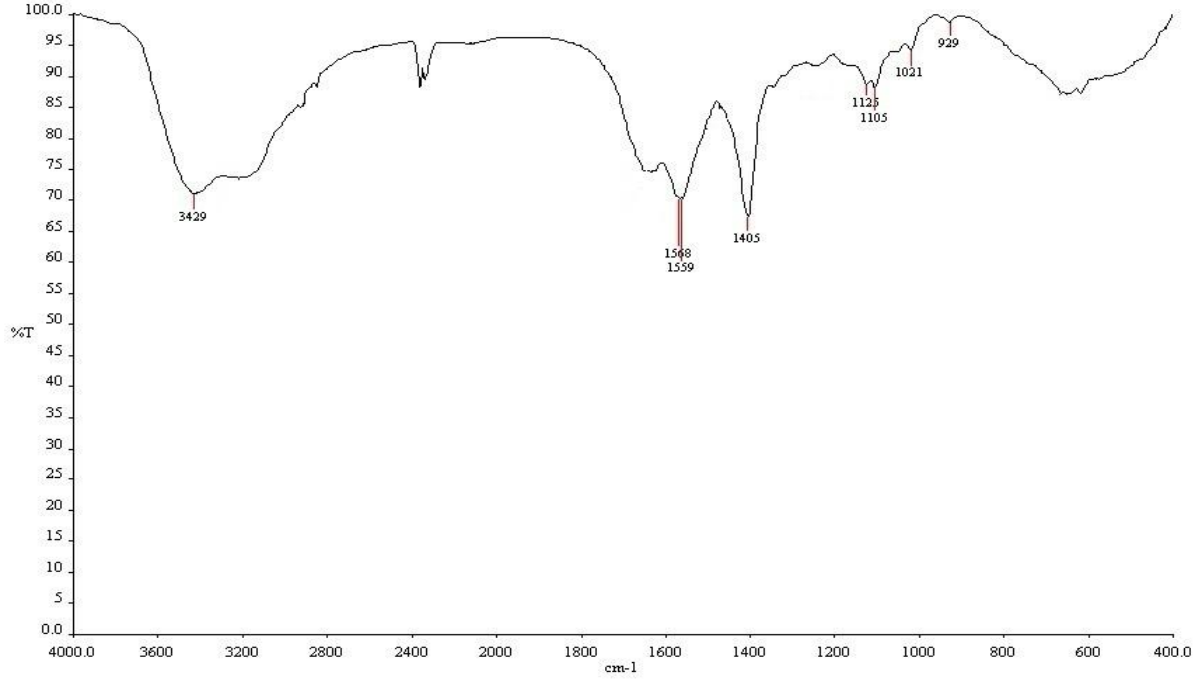


**Fig: 4 FT-IR spectrum of crude chitosan from *P. stylifera* shrimp shell waste**

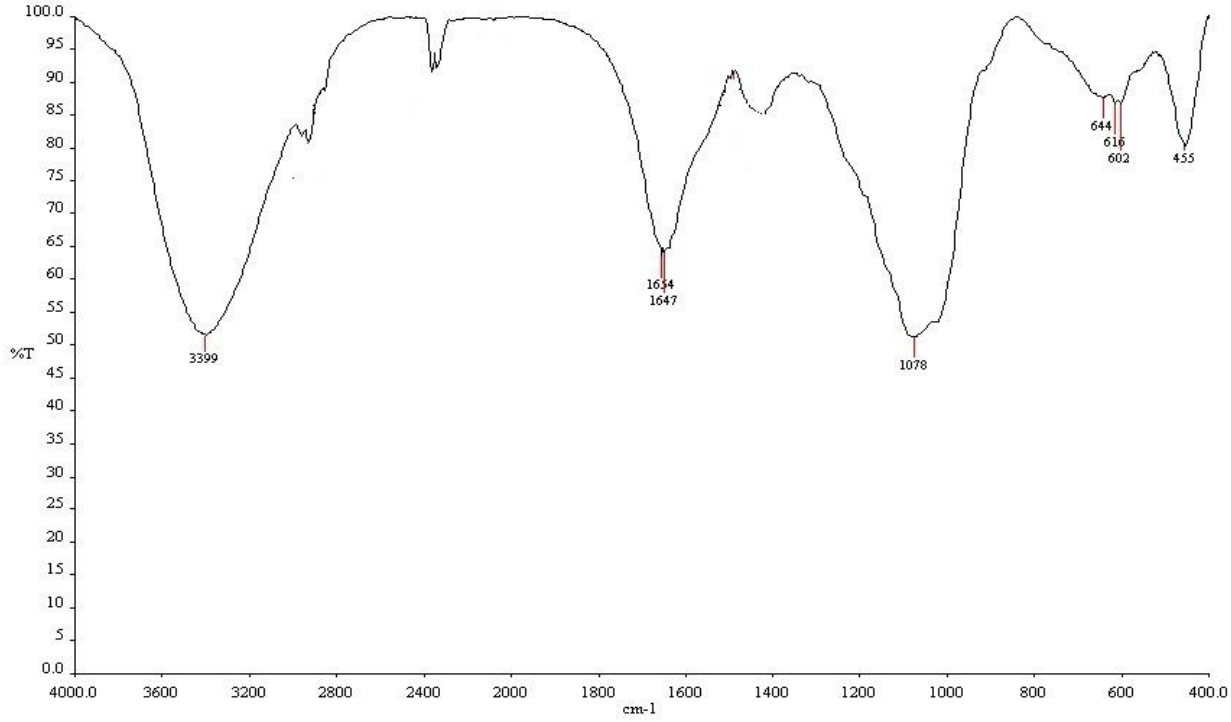


**Fig: 5 FT-IR spectrum of water soluble chitosan from *P. stylifera* shrimp shell waste**

**Table-2: Wave length of the main bands obtained from the standard chitosan and Water soluble chitosan from *P. stylifera* shrimp shell waste**

| **Vibration mode Chitosan Shell** | **Std. chitosan** | **Crude Chitosan** | **Water soluble chitosan** |
|---|---|---|---|
| Structural unit | 3436 | 3429 | 3399 |
| (-NH2) Amide II | 1636 | 1568 and 1559 | 1654 and 1647 |
| $PO3\ 4^-$ | 1322 | 1405 | --- |
| $PO4\ 3^-$ | 1019 | 1105 and 1021 | 1078 |
| (NH) Amide III | 894 | 929 | - |
| NH-out of plane bending | 778 | -- | 644 |

**Table-3: Antibacterial activity of the crude chitosan and water soluble chitosan from *P. stylifera* shrimp shell waste:**

| | Inhibition Zone (mm) | | | | | |
|---|---|---|---|---|---|---|
| Microorganisms | Crude chitosan | | Water soluble chitosan | | Positive control | Negative control |
| | 500µg/ml | 1mg/ml | 500µg/ml | 1mg/ml | | |
| *E. coli* | 5.2 | 8.4 | 7.3 | 10.4 | 10 | - |
| *P. aeruginosa* | 4.3 | 6.1 | 7.5 | 8.4 | 6 | - |
| *K. oxytoca* | - | 3.2 | 4.0 | 7.3 | 5 | - |
| *S. aureus* | 6.4 | 8.9 | 10.2 | 18.3 | 17.6 | - |
| *S. pneumoniae* | 2 | 4.3 | 5.1 | 6.2 | 6 | - |
| *K. pneumonia* | - | - | - | 4.4 | 4.5 | - |
| *S. typhi* | 1.4 | 4.2 | 4.7 | 6.6 | 6.8 | - |

-, No activity was observed