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COMPOUND ISOLATION OF *PORTUNUS SANGUINOLENTUS* CHITOSAN FABRICATES CRAB SHELL

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Abstract:

Isolation of chitin and chitosan from natural sources are widely known as they can be used in many applications. Extraction of chitin and chitosan are carried out in exoskeleton crab shell Portunus sanguinolentus. Chemical treatments such as demineralization and deproteinization processes are used for chitin extraction and followed by deacetylation process of chitin, which is to produce its derivatives that known as chitosan. Characterization of chitin and chitosan has been done using Fourier Transform Infrared (FTIR) Spectroscopy, Nuclear Magnetic Resonance (NMR) and Gas chromatography and Mass Spectrum (GCMS) analysis and the functional group analysis have indicated the presence of a carbonyl group and most effective ester groups. The FT-IR spectrum was used to identify the functional groups of the active components peaks as 567 cm^{-1}(C-Br), 711cm^{-1} (C-Br),871(C-F)cm^{-1},1028 cm^{-1},1066 cm^{-1}, 1151 cm^{-1}(C-F) which is indicate alkyl halide (CF), 1647 cm^{-1}, 1727 cm^{-1} (C=O), which possible of ester groups and 2802 cm^{-1}, which indicate aliphatic alkane $(CH_2)_n$, 3304 cm^{-1} (H-H stretch, strong bonded indicated the presence of chitin and chitosan. Preliminary results based on the future study like GCMS and NMR.

Keywords: *Portunus sanguinolentus, FTIR, NMR, GCMS, Functional group.*

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INTRODUCTION:

Chitosan attained from a characteristic polymer chitin has an antibacterial element. As a polycationic polymer, chitosan is an ecological benevolent material in light of its biodegradability. Nontoxic and antibacterial highlights of chitosan make it usable for some, regions identified with human wellbeing [1]. Chitosan is utilized as metal nanoparticle-chitosan material in biomedical applications in view of its points of interest of biodegradability, antibacterial properties, and superb chelating operator. Both of Ag and chitosan are antibacterial specialists so chitosan-Ag nanoparticle composite material has a more antibacterial impact [2]. Chitin extraction process is basically composed of demineralization and deproteinization stages. Chemical and biological methods may be employed for the two major stages of this extraction process. In the chemical method, acid and alkaline reagents are employed for the removal of inorganic material and hydrolysis of protein, respectively. On the other hand, the biological method makes use of enzymatic extracts or isolated enzymes and biological fermentation. The product of this method, however, has significantly low quality compared with the chemical method [3]. Biomaterials are those non-living materials used in the medical, biomedical and other fields, aiming to interact with the biological system. Chitin extraction process is basically composed of demineralization and deproteinization stages. Chemical and biological methods may be employed for the two major stages of this extraction process. In the chemical method, acid and alkaline reagents are employed for the removal of inorganic material and hydrolysis of protein, respectively. On the other hand, the biological method makes use of enzymatic extracts or isolated enzymes and biological fermentation. As of late, a few sorts of research have been done going for new materials fit for being related with different substances that advance bone development, particularly biopolymers, specifically, chitosan, which presents awesome potential in the repair of bone deformities, in connection to the restrictions of different biomaterials [4].

Asia has abundant species of medicinal and aromatic plants and traditional medicines have practiced in Asia since ancient times [6] the development of reconstructive surgery, cardiac surgery, transplantation, and dentistry would not have been possible without progress in the field of material science, chemistry and technology polymers for biomedical and bioengineering materials. One of the new and promising biomaterials being used in dentistry is chitosan Silver has been commercially employed as a broad-spectrum antibiotic agent, and it is not yet associated with antibiotic resistance like other pharmaceuticals [5]. Marine biotechnology is the science in which marine organisms are used in full or partially to make or modify products, to improve plants or animals or to develop microorganisms for specific uses. With the help of different molecular and biotechnological techniques, humans have been able to elucidate many biological methods applicable to both aquatic and terrestrial organisms [7]. Nowadays conventional medicinal plants are fetching popular for use in disease behavior, preventive medicine, and health encouragement and normally categorize as an alternative or harmonizing medicine [8]. Medicine standards of the plants are due to the compounds that produce a definite physiological action on the human body and are called chemical constituents. Free radicals are associated with several diseases including cancer, diabetes mellitus, and arthritis, aging and liver disorder [9].

MATERIALS AND METHODS:

Collection of crab shells

The *Portunus sanguinolentus* were collected from the fish market at Chidambaram (Lat. 11º.39'N and Long. 79º 69'E) near Vandigate (Figure 1).

Figure 1. *Portunus sanguinolentus* crab shell image.

The skeleton was placed in Ziploc cover and frozen overnight and then subsequently cut into smaller pieces using a meat tenderizer. Wet samples of 10 g of crushed crab exoskeletons were placed on foil paper and measured using a metal balance, with five replications. The labeled samples were then oven-dried at 70ºC for four consecutive days until they obtained constant weight. The dry weight of the samples was then determined, and the moisture content was also measured based on the differences between wet and dry weight.

Fourier Transforms Infrared spectra

Ultraviolet (UV) spectrums were recorded on Shimadzu UV-170 spectrophotometer. One milligram of the sample was dissolved in 10 ml of water and the spectra were recorded at 200–400nm range. The

infrared spectra were recorded on a Shimadzu IR-470 Model. The spectra were scanned in the 400 to 4000 cm-1 range. The spectrum was obtained using potassium bromide pellet technique. Potassium bromide was dried under vacuum at 100°C for 48 h and 100mg of KBr with 1mg of the sample was taken to prepare a KBr pellet. The spectrum was plotted as intensity versus wave number [18].

GC-MS Analysis

GC MS analysis was carried out on Shimadzu 2010 plus comprising an AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0.32mm, column length is 30m, column thickness 0.50µm), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml /min and an injection volume of 0.5 µl was employed (split ratio of 10:1) injector temperature 270ºC; ion-source temperature 200ºC. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0 [19].

Nuclear Magnetic Resonance

The bioactive compound was subjected to ^{1}H and $^{13}CNMR$ (500 MHz, Brucker Biospin, Switzerland) studies. The bioactive compound was dissolved (3mg for 1HNMR) in 3 ml of chloroform and analyzed, by Nuclear Magnetic Resonance (NMR)[20].

RESULTS AND DISCUSSION:

Fourier transforms infrared (FTIR) spectroscopy is a technique that is used to determine the vibration of functional groups in chitin and chitosan. The FTIR spectra of chitin and chitosan of *P. pelagicus* were examined as shown in (Figure 2) Spectrophotometric studies were made using a Shimadzu FT-IR-8501 spectrophotometer in the range 4600-400 cm-1 and resolution ± 4cm-1. The total samples were pelletized with KBr in the weight ratio 1/100.Spectric of micro granules which absorbed different quantities of Ag+ ions -0.125 mMol/g (initial adsorptive concentration 50 mg/dm3 and 1.13 mMol/g (Initial Adsorptive Concentration 500 mg/dm3). The present study compared to The FT-IR spectrum of the standard chitosan reported 8 major peaks lying between 523.90 and 3434.37cm-1; whereas the FT-IR spectrum of the chitosan sample from mangrove crab of *S. plicatum* recorded 6 peaks between 408.04 and 3914.81cm-1[10]. The spectra were presented in the full range of frequencies from 4400-400 cm-1 and the range corresponding to local symmetry 1500 – 1200 cm-1. In the IR spectrum, a broadband 3600 – 310 cm-1 is observed its shape changes toward lower frequencies it becomes more symmetric which indicate the presence of OH and NH2 groups. The studies in the literature about FTIR spectroscopy related with chitosan showed some characteristic peaks, which are at 2940 cm-1 (–CH3, –CH2), 1655 cm-1 (C=O stretch vibration of secondary amide I band), 1555 cm-1 (N–H bending vibration of amide II band), 1570 cm-1 (N–H bending vibration of primary amides) and 1070 cm-1 (C–O stretching) [11].

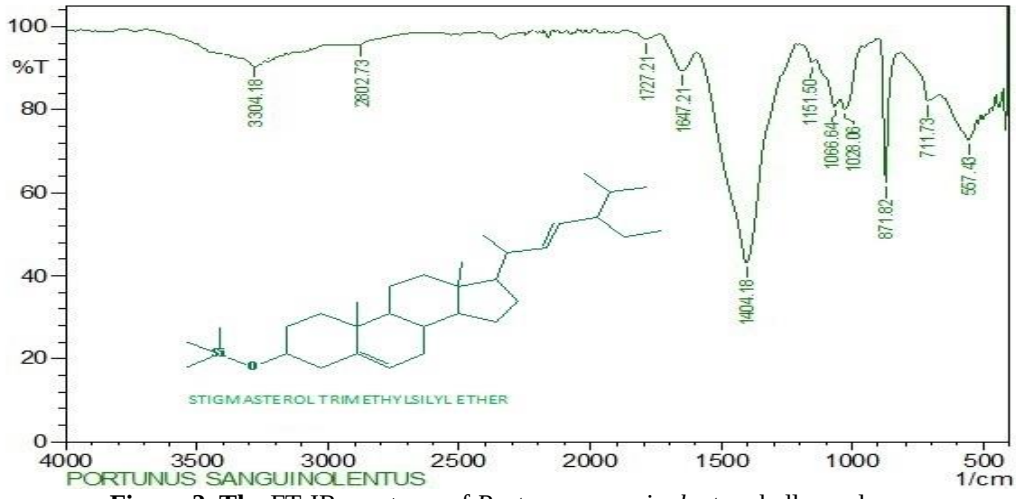

Figure 2. The FT-IR spectrum of *Portunus sanguinolentus* shell powder

In the present investigation observed the structure of active polar solvent methanolic third fraction have more active oxidative scavenge from crab shell extract compound was further elucidated by GC-MS, The main compound relative intensity of crab shell which is exhibited molecular adduction peak at m/z 484.9 and Retention time 43.05 (Figure 3) The mass spectrum of compound from crab shell was found to be identical may be active compound to Stigmasterol Trimethylsilyl Ether. The structure of the compound was identified as Stigmasterol Trimethylsilyl Ether and the structure of the compound was also confirmed from the literature report followed and confirmed made standard methods (Barber et al., 1988).

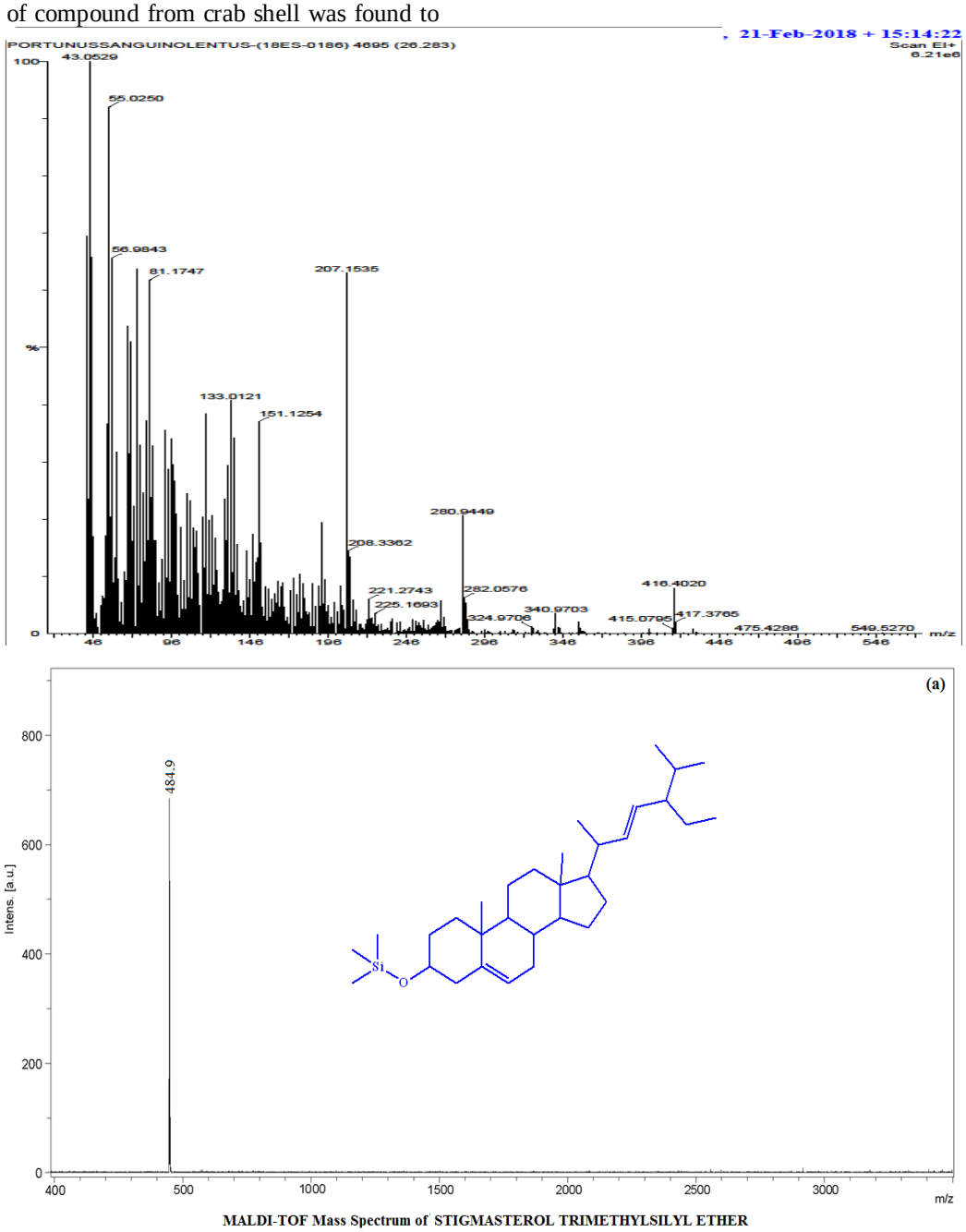

Figure 3. Gas Chromatography and Mass Spectrometry of derived compound

The crab shell powder was observed from the highest peak value of crab shell powder was analyzed by GC-MS. The chemical compositions of the extracts were ascertained using the data obtained from the elemental and functional group analysis as well as FT-IR and NMR spectral studies. The NMR (400 MHz, CDCl3) spectra of highest peak was subjected to the NMR analysis for 1H and 13C NMR, it was confirmed that the major active groups present in the fractions producing signals in the upfield level in the range of delta values from 2.00 to 14.21 - 1HNMR and 24.32 to 112.75 - 13C NMR. The 1HNMR spectra (Figure 4 and Figure 5) of Crab shell showed the presence of Ester groups. Anal. calc. (C32H56OSi): C, 84.91; H, 4.37; O, 7.39; Si, 5.79. Found: C, 84.89; H, 4.23; O, 7.30; Si, 5.70.

Snow crab shells have a high economic potential because their mean weight is similar to the mean weights of other whole crabs, such as blue crab, swim crab [12], or green crabs [13]. [14] Indicate the nutritional value of crustaceans as potential candidates for use in diets. Compared to our snow crab shells, the reported moisture and protein content in meat were higher; however, it is not the same for total lipids and ash content. In our snow crab shells, the lipid content and ash are higher than the crustacean meat, and it is similar to that found in snow crab by-products and for edible viscera [15] and hepatopancreas [17]. It is a test of the nutritional value of such by-products, with values equal to or greater than edible crustaceans, which are discarded.

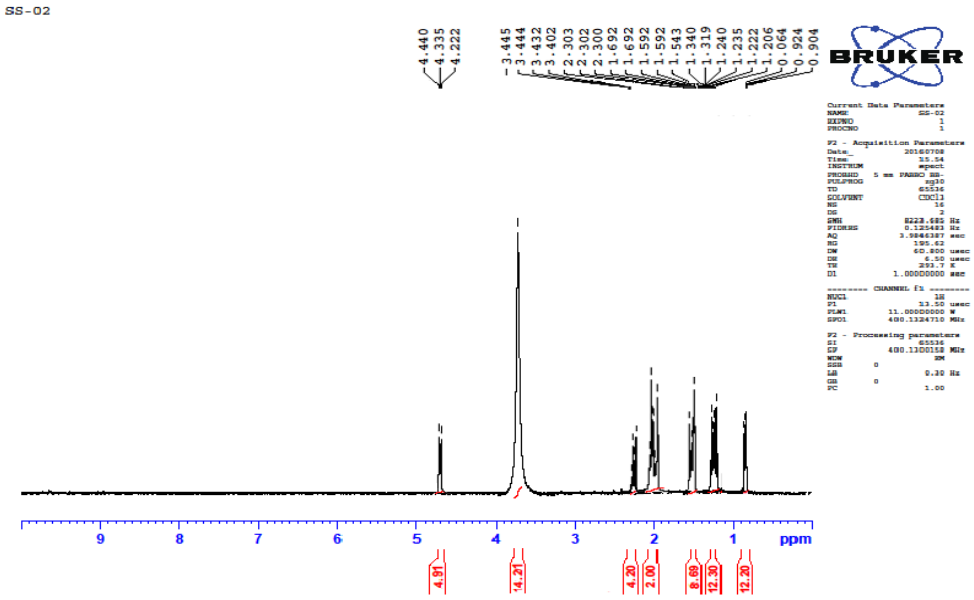

Figure 4. NMR H^1 Spectrum of crab shell derived compound

1H NMR (400 MHz, CDCl3): d 0.21 (s, 9H), 0.90 (t, 3H), 0.91 (d, 6H), 1.04 (s, 3H), 1.12 (d, 1H), 1.10 (d, 3H), 1.39 (d, 1H), 1.44-1.60 (m, 19H), 1.86-2.23 (m, 5H), 2.33 (m, 1H), 3.26 (m, 1H), 5.37 (t, 1H), 5.48 (d, 2H). 13C NMR (400 MHz, CDCl3): d 3.9, 12.4, 19.2, 20.1, 21.3, 25.5, 26.5, 28.8, 31.9, 32.6, 37.2, 37.8, 40.0, 43.0, 50.1, 52.3, 56.5, 75.4, 121.6, 129.4, 138.2, 140.9, MS: m/z.484.3 [M¬+].calcd. 484.2

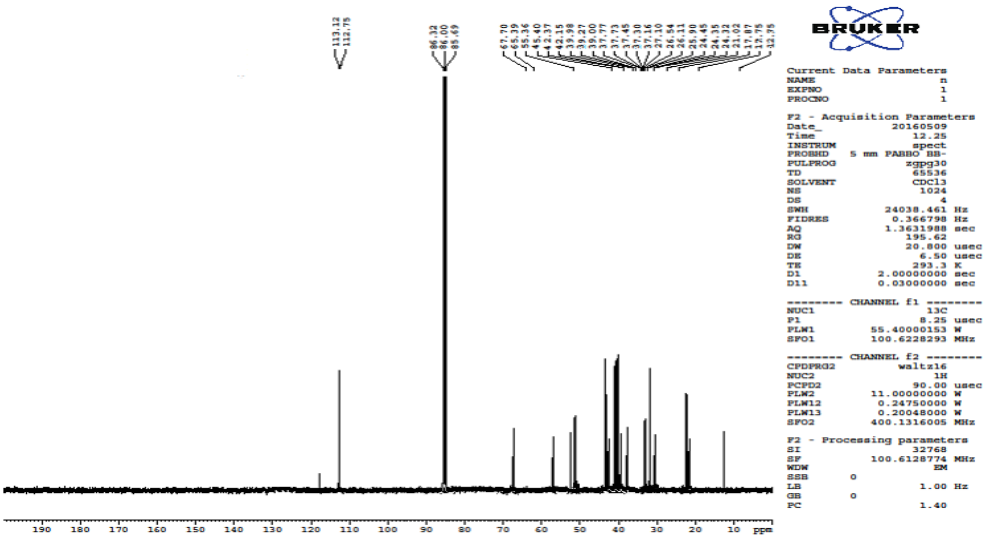

Figure 5. NMR C ^{13}spectrum of crab shell powder derived compound

CONCLUSION:

The importance of chitin and chitosan resides in their biological (biodegradability, biocompatibility, and nontoxicity) and physicochemical properties (degree of acetylation and molecular mass). Recently, these properties are widely applied in agriculture, medicine, pharmaceutics, food processing, environmental protection, and biotechnology. The extraction of chitin by a chemical method using concentrated acids and bases in order to deproteinize and to demineralize crab shell waste at high temperature can deteriorate the physicochemical properties of this biopolymer and consequently its biological properties, which results in products of varying quality that are neither homogeneous nor reproducible. Nowadays, a new method based on the use of lactic acid bacteria and/or proteolytic bacteria has been used for chitin extraction.

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