

Comparative study on antimicrobial activity of commercial and extracted chitin and chitosan from *Marsupenaeus japonicus* shells

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ABSTRACT

Chitin was extracted from the skeleton of *Marsupenaeus japonicus* shrimp shell waste with a yield of 27.7% of the net weight through three steps namely; deproteinization, decolourization and demineralization. After deacetylation process of extracted chitin, chitosan was produced and represented 55.1% of the extracted chitin. Scanning electron microscope and Fourier Transform Infrared spectroscopy (FTIR) analyses were conducted to characterize the obtained chitin and chitosan. The FTIR analysis revealed different absorption bands within 4000-400 cm^{-1} range; by comparison these bands were found to be similar to those of commercial chitin and chitosan and confirmed partial deacetylation in the produced chitosan. Moreover, the antimicrobial activity of commercial and extracted chitin and chitosan were examined against different pathogenic Gram positive, Gram negative bacteria and fungi using agar well diffusion test. Results showed that there was no significant difference $p < 0.05$ in the antimicrobial activity of the extracted compounds and commercial ones against test pathogens as the extracted compounds recorded inhibition zones ranged from 12 to 23 mm in diameter while the commercial compounds recorded inhibition zones of diameters ranged from 12 to 22 mm only; with *Staphylococcus aureus* ATCC 25923 being the most affected microbe with inhibition zone of 23 mm produced by extracted chitin, while the lowest zone of inhibition was recorded against *Enterococcus faecalis* ATCC 29212 with only 12 mm by commercial and extracted chitosan.

INTRODUCTION

Crustacean processing produces about 40% of shell waste (Gildberg and Stenberg, 2001). The global annual production of shell waste is estimated to be 1.44 million metric tons dry weight (Rodde *et al.*, 2008; Kean and Thanou, 2011). Since biodegradation of this waste is very slow, accumulation of large quantities has become a major concern in the seafood processing industry. Therefore, the use of this waste to produce renewable products such as biopolymers is a dual-purpose opportunity. The Crustacean shells consist mainly of chitin, proteins, lipids, pigments and trace elements. Chitin, calcium carbonate and proteins account for about 90% of the dry weight of the shell (Rodde *et al.*, 2008; Benhabiles *et al.*, 2012).

The extraction of chitin from the shells is achieved in three steps: 1) deproteination, 2) removal of lipids and pigments and 3) demineralisation. The chitin extraction steps and its subsequent conversion to chitosan can be carried out chemically or by using biological methods, such as microbial fermentation and enzymatic reactions (Acharya *et al.*, 2005; Rinaudo, 2006).

Chitosan is a technologically important polysaccharide biopolymer. Chemically, it is a high molecular weight linear polycationic heteropolysaccharide consisting of two monosaccharides, N-acetyl-D-glucosamine and D-glucosamine, linked together by β -(1 \rightarrow 4) glycosidic bonds. Chitosan is primarily produced from chitin by exhaustive alkaline deacetylation, this process involves boiling chitin in concentrated alkali for several hours. Since this N-deacetylation is almost never complete, chitosan is considered as a partially N-deacetylated derivative of chitin (Fouad, 2008).

Moreover, early research described the antimicrobial potential of chitin, chitosan, and their derivatives (Chen *et al.*, 1998; Shahidi *et al.*, 1999). Numerous bacteria and fungi are highly pathogenic causing various infectious diseases (Chistiakov *et al.*, 2007). The increasing economic and social concern to decrease the use of antibiotics and other therapeutic chemicals has encouraged more environmentally friendly approaches to control diseases (Torrecillas *et al.*, 2007). Interestingly, chitin and chitosan have been investigated as antimicrobial agents against a wide range of target organisms like algae, bacteria, yeasts and fungi in experiments involving *in vivo* and *in vitro* interactions in different forms (solutions, films and composites) (Goy *et al.*, 2009).

Generally, in these studies the chitosan is considered to be a bactericidal (kills the live bacteria) or bacteriostatic (hinders the growth of bacteria but does not imply whether or not bacteria are killed), often with no distinction between activities. Recent data in literature has the tendency to characterize chitosan as bacteriostatic rather than bactericidal (Coma *et al.*, 2002; Toan *et al.*, 2013) and it has been shown to inhibit growth of several fungi and bacteria, especially pathogens (Islam *et al.*, 2011). Although, various theories have been proposed to explain the mechanism of the antibacterial activity of chitosan, intracellular leakage hypothesis is widely accepted (El-Badawy *et al.*, 2003; Kong *et al.*, 2010). Positive charge of chitosan binds to the negative charge on the surface of the bacterial cell causing altered membrane permeability which leads to leakage of intracellular constituents causing cell death. Therefore, the antibacterial activity of chitosan is suggested to be limited to acidic condition as the positive charge of chitosan amino group is lost at neutral pH (Qi *et al.*, 2004; Liu *et al.*, 2004).

Therefore, the objective of this study was to extract and characterize chitin and chitosan from shrimp (*Marsupenaeus japonicus*) shells and to test the possibility of using them as safe and eco-friendly antimicrobial agents. Moreover, the current study aimed to compare this activity with commercial chitin and chitosan.

MATERIALS AND METHODS

All chemicals used in this study were of analytical grade and purchased from local suppliers. Shrimp shells were obtained from fish market, Alexandria.

Pretreatment of shrimp shells for chitin extraction

Shrimp shell waste were collected and washed three times thoroughly using tap water to remove the salt on its surface, followed by washing with distilled water then spreading on blotting paper to remove excess water. They were dried by hot air oven at 70°C overnight. All dried shrimp shells were milled using a laboratory hammer mill in order to obtain a particle size less than 0.5 mm.

Determination of *M. japonicas* shell chemical composition

The chemical composition of *M. japonicas* shrimp shells was determined by Plant Biochemistry Department, Agricultural Research Center, Giza, Egypt. Moisture content was considered as the losses in mass from a sample (1 g) after drying at 100°C ± 2°C. The residual mass in this sample was heated at 600°C ± 10°C to determine the ash content. The organic matter was calculated through the difference between 100% and the sum of moisture content and ash values.

Nitrogen was measured by Kjeldahl method using the distiller-digester (Yokoyama and Guimarães, 1975). Once the nitrogen content has been determined it is converted to a protein content using the appropriate conversion factor.

Protein% = F x N% where F (Conversion factor) = 6.25 and N% is the nitrogen percent.

For lipid contents, 2 g of sample was extracted with ethyl ether in Soxhlet extractor for 5 h. The solvent was evaporated and the residual mass on the balloon was considered lipids (Pádua *et al.*, 2004). Subtraction of the sum of moisture content, protein, lipids, chitin and ash values from 100 was the carbohydrate contents in percentage (Pádua *et al.*, 2004).

Chitin extraction

In order to obtain purified chitin, it must be separated from the proteins, minerals and other components. This separation was achieved in three steps according to Mohammed *et al.* (2013) with slight modifications as follows:

- 1- Deproteinization by 5% NaOH (w/v 1:8) at 60°C for 2 h then the sample was washed with distilled water and dried at 60°C until constant weight.
- 2- Decolourization by treatment with acetone (w/v 1:10) for 24 h at room temperature followed by bleaching with 0.315% NaOCl (w/v 1:10) for 5 min then the sample was washed with distilled water and dried at 60°C until constant weight.
- 3- Demineralization of the produced material by 0.5 or 1% HCl solution (w/v 1:4, 1:10) for 24 h at 25°C to dissolve calcium carbonate.

The sample was washed with distilled water to remove CaCl₂ and other water soluble impurities. At the end, the extracted chitin was in the form of light brown powder. The shrimp shell chitin contents were calculated as the weight difference of the obtained chitin and the raw dried shrimp shell waste (Mohammed *et al.*, 2013).

Deacetylation of chitin for chitosan production

Chemical deacetylation was achieved by treatment of extracted chitin with 50% NaOH solution (w/v 1:5) at elevated temperature and then boiled at 100°C for 2 h on a hot plate. After the reaction the material produced was washed several times with distilled water until near to neutral pH and dried at 60°C in a vacuum oven until constant weight. The chitosan obtained had a white creamy form (Muzzarelli and Rochetti, 1985, Mohammed *et al.*, 2013).

Characterization of the extracted chitin and chitosan:

Fourier Transform Infrared Spectroscopy (FTIR) analysis

Chitin and chitosan samples were ground to a very fine powder with KBr and dried thoroughly. The dried mixture was pressed under vacuum in a mould to form a

KBr disc containing the sample. The FTIR spectra were recorded using a Perkin Elmer FTIR Spectrometer according to Kumirska *et al.* (2010) over the frequency range of 4000-400 cm^{-1} .

Scanning electron microscopy

The extracted chitin and chitosan samples were examined and photographed using SEM (JSM-5300 – JEOL, Japan), available through Faculty of Science, Alexandria University.

Antimicrobial activity assay

Five microbial pathogens namely; *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739 and *Candida albicans* ATCC 10231 were grown over night in nutrient broth at 37°C.

Gels were prepared by dissolving one gram of chitin or chitosan in 99 ml of 1% glacial acetic acid (pH 4) to produce gels with 10 mg/ml final concentration. The gels were homogenized for 6 h at 60°C under moderate stirring conditions. A commercial chitin (Loba chemie, India- Mwt: 400,000) and chitosan (Acros Organics, USA- Mwt: 100,000-300,000) were dissolved under the same conditions for comparison. In addition a 1% glacial acetic acid was serving as a control. After homogenization all preparations pH were adjusted to 5.6 ± 0.2 (slightly acidic). The well diffusion method (Rayn *et al.*, 1996) was used to evaluate the antimicrobial activity of all preparations against the indicated pathogens, separately. Briefly, each pathogen (1% v/v) was inoculated in nutrient agar plates then, wells (8 mm in diameter) were punched onto the surface using a sterile borer. A 150 μL of each preparation were added separately to wells. Plates were stored at 4°C for 30 min to allow diffusion. All plates were prepared in triplicates and they were incubated at 37°C for 24 h before examination. A clear inhibition zone at least 10 mm in diameter was recorded as positive.

RESULTS AND DISCUSSION

In this article chitin was extracted according to the method mentioned before from the skeleton of *M. japonicus* shrimp with 27.7% of net weight. This result with the other chemical compositions of the shrimp shells are illustrated in Table 1.

Table 1: Chemical composition of *M. japonicus* shrimp shells

Constituent	% to dry wt.
Moisture content	21.1
Ash content	5.2
Organic matter:	73.7
Chitin	27.2
Crude protein	16.3
Carbohydrate	19.3
Lipids	10.9

Abdou *et al.* (2007) extracted chitin from six different local sources in Egypt. They converted the obtained chitin into the more useful soluble chitosan by steeping into solutions of NaOH of various concentrations for extended periods of time, then the alkali chitin was heated in an autoclave which dramatically reduced the time of deacetylation. So, in this study, we exposed the chitinous wastes collected from shrimp to NaOH, followed by HCl to obtain treated chitin shells (Ibrahim *et al.*, 2015).

The chitosan yield was 55.1% of the produced chitin extracted from the total exoskeleton taken. By comparison, Puvvada *et al.* (2012) collected the crude chitin from exoskeleton of *Triops longicaudatus* and *Triops cancriformis* specimens which are then processed to obtain chitosan. The chitosan yield was found to be 35.49% and it was analyzed for its physiochemical parameters. On the other hand, Paul *et al.* (2014) focused on the purification of chitosan from chitin isolated from *Fenneropenaeus indicus* for pharmaceutical industry. However, Paul *et al.* (2014) found that the chitosan yield was 57.69%.

As shown in Fig. 1, the extracted chitin and chitosan from the *M. japonicus* shrimp shells, chitin can be seen as a light brownish powder, while the produced chitosan obtained by chitin deacetylation exhibited a creamy color. On the other hand, Fig. 2 indicates scanning electron microscopy image of the extracted chitin and chitosan.

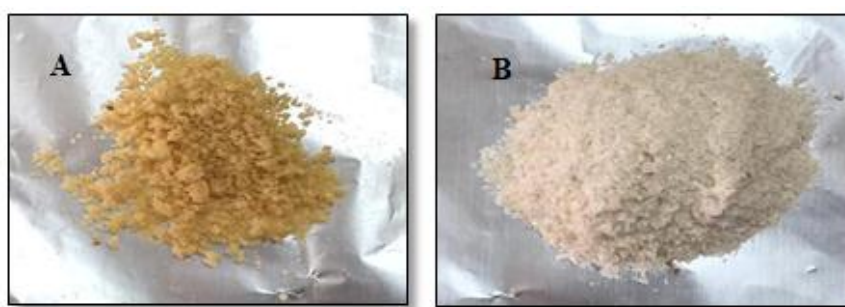


Fig. 1: Crude chitin (A) and chitosan (B) extracted from *M. japonicus* shells.

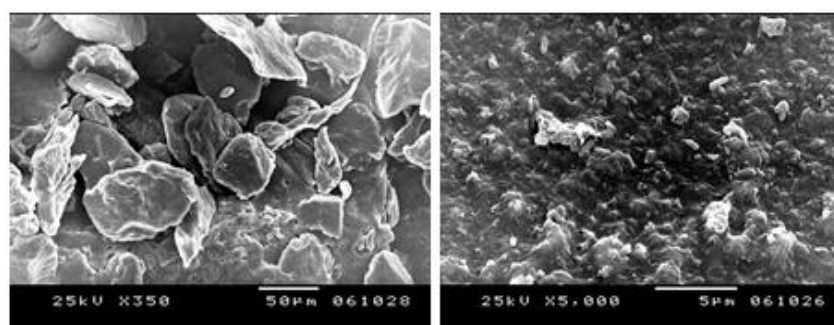


Fig. 2: Scanning electron microscopy image of Chitin (left) and Chitosan (right) extracted from *M. japonicus* shells.

Data in Fig. 3 and Table 2 demonstrates the FTIR spectrum of extracted chitin in the range of $4000\text{--}400\text{ cm}^{-1}$. Bands between 1200 and 1100 cm^{-1} are assigned to C-C and C-O modes including a shoulder due to C-O-C of the glycosidic linkage (1153 cm^{-1}). The spectra for the OH bending modes and sugar OH stretching were observed at 3256 cm^{-1} . However, the absorption bands of produced chitin were identical to those of standard chitin.

These findings were in the agreement of results obtained by Rinaudo, (2006) and Kumirska *et al.* (2010). They confirmed that Amide I band, which is responsible for the splitting of wave numbers between 1600 to 1500 cm^{-1} , corresponds to the inter-sheet hydrogen bonding due to the hydroxymethyl group that can be associated to the band peak at 1630 cm^{-1} and the intra-sheet hydrogen bonding mat the C=O stretching region with wave number 1660 cm^{-1} . This inter- and intra-sheet hydrogen bonding is characteristic of chitin which gives chitin's highly insoluble property.

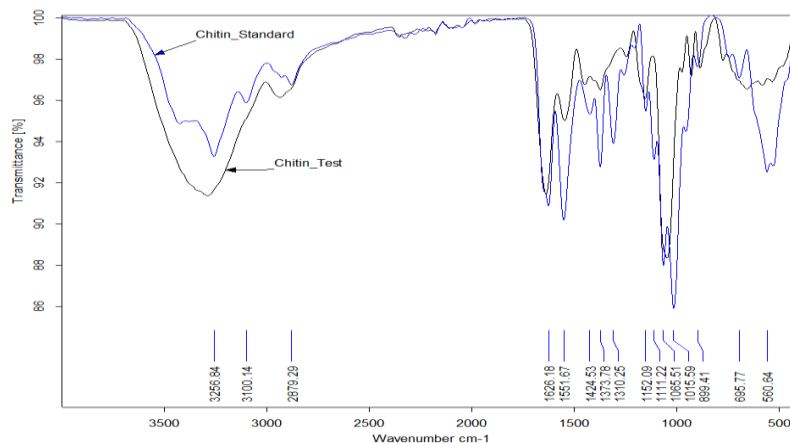


Fig. 3: FTIR of chitin extracted from *M. japonicus* shells compared to commercial chitin.

Table 2: Wave length of the main bands obtained by the FTIR for both commercial and extracted chitin

Frequency (cm ⁻¹)	Assignments
3100	Glycopyrenose
1065	Carbohydrate backbone
1424	Amide III
1551	Amide I
1626	N-acetyl ester bonds, Amide II
890	Amino peak, alpha chitin

In addition, different absorption bands within 4000-400 cm⁻¹ range were presented in the FTIR spectra of chitosan as shown in Fig. 4 and Table 3. These bands were compared to those of standard chitosan. The absorption bands of produced chitosan were identical to those of standard chitosan. The bands ranged from 3425-2880 cm⁻¹ related to (N-H) stretching frequency of NH₂ and (OH) groups which present in chitosan. The band at 1646 cm⁻¹ suggests effective deacetylation band at 1650 cm⁻¹ represent acetylated group which due to C=O.

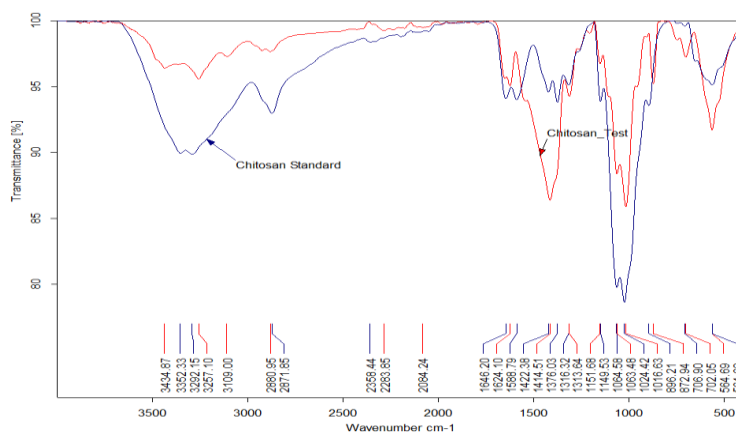


Fig. 4: FTIR of chitosan extracted from *M. japonicus* shells compared to commercial chitosan.

Table 3: Wave length of the main bands obtained for both commercial and extracted chitosan

Vibration modes	Chitosan	
	Standard	Produced
NH out-of- plane blending	706.90	702.05
Ring stretching	896.21	872.94
CO stretching	1024.42	1016.63
CH ₂ bending and CH ₃ deformation	1422.38	1414.51
Amide II band	1588.79	-----
Amide I band	1646.20	1624.10
CH stretching	2871.85	2800.95
NH stretching	3292.15	3257.10

In general, commercial chitosan shows bands at 3000-3500 cm⁻¹ (NH bond) and at 1400-1650 cm⁻¹ (C=O bond) (Chatterjee *et al.*, 2005). Paul *et al.* (2014) revealed that the major absorption band is observed between 1220-1020 cm⁻¹ which represents the free amino group (-NH₂) at C2 position of glucosamine, a major group present in chitosan. Further data obtained by Puvvada *et al.* (2012) revealed that their sample showed the absorption bands at the various peaks, which is similar to standard chitosan. This also shows the confirmation of chitosan (Puvvada *et al.*, 2012).

In addition, George *et al.* (2011) obtained FTIR spectra of chitosan that showed a broad absorption band in the range 3000-3500 cm⁻¹ attributed to O-H stretching vibrations. The peaks around 2885, 1650, 1589, 1326 and 1080 cm⁻¹ in the FT-IR spectrum of chitosan were due to the stretching vibrations of aliphatic C-H, Amide I (-NH deformation of -NHCOCH₃), Amide II, Amide III and C-O-C, bonds respectively. However, these are characteristics of the chitosan polysaccharide (Radhakumary *et al.*, 2003). Similar FTIR spectrum of chitosan obtained by Mohammed *et al.* (2013).

The antimicrobial activities of extracted chitin and chitosan compared to corresponding commercial ones were detected. They were expressed as the diameter in millimeters of inhibition zone. Results in Fig. 5 and Table 4 showed that there was no significant difference $p < 0.05$ in the antimicrobial activity of the extracted compounds and commercial ones against test pathogens as the extracted compounds recorded inhibition zones ranged from 12 to 23 mm in diameter while the commercial compounds recorded inhibition zones of diameters ranged from 12 to 22 mm, with *Staphylococcus aureus* ATCC 25923 being the most affected microbe with inhibition zone of 23 mm produced by extracted chitin, while the lowest zone of inhibition was recorded against *Enterococcus faecalis* ATCC 29212 with only 12 mm by commercial and extracted chitosan. Surprisingly, the inhibition zones produced by chitin either extracted or commercial recorded higher values than those by both extracted and commercial chitosan.

On the other side, both chitin and chitosan were more effective against bacteria than fungi. The degree of deacetylation has a major effect on the antimicrobial activity as it is determinant in the charge development and solubility of chitosan and increasing the degree of deacetylation will increase the free amino groups in the produced chitosan which leads to higher antimicrobial activity, as the -NH₂, -OH groups are considered the main reactive site in chitosan.

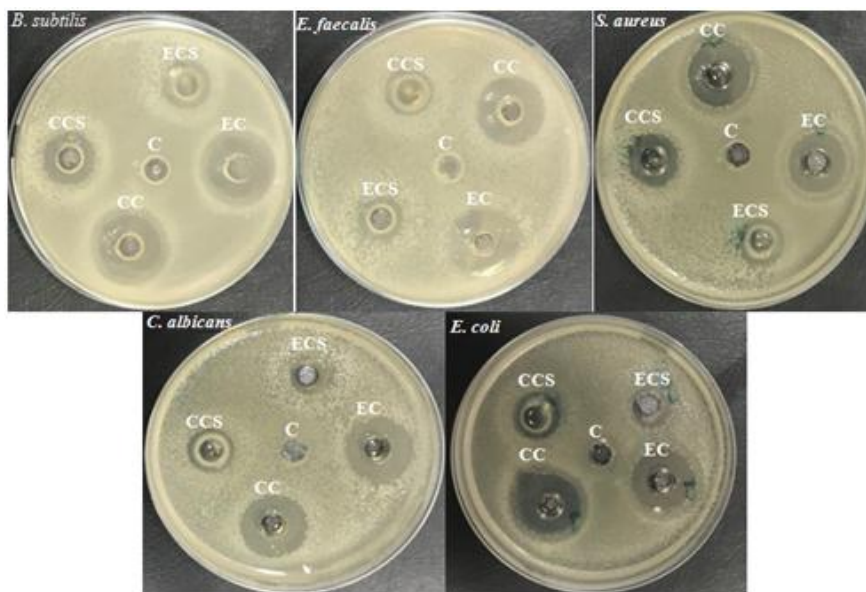


Fig. 5: Antimicrobial activity of extracted chitin and chitosan against reference strains compared to commercial chitin and chitosan (EC: Extracted Chitin, ECS: Extracted Chitosan, CC: Commercial Chitin, CCS: Commercial Chitosan).

The effectiveness of chitin and chitosan as antimicrobial agents is well documented in many studies against both bacteria and fungi. For example, Benhabiles *et al.* (2012) detected the antimicrobial activities of chitin and chitosan isolated from shrimp (*Parapenaeus longirostris*) shell waste against four Gram-positive and seven Gram-negative bacteria. Their data confirmed that chitin exhibited a bacteriostatic effect on Gram-negative bacteria namely, *E. coli* ATCC 25922, *Vibrio cholerae*, *Shigella dysenteriae*, and *Bacteroides fragilis*. The chitosan exhibited a bacteriostatic effect on all bacteria tested (*E. coli*, *Pseudomonas aeruginosa*, *V. cholerae*, *S. dysenteriae*, *Prevotella melaninogenica*, *Enterobacter agglomerans*, *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus* and *B. fragilis*), except *Salmonella typhimurium*. In addition, Paul *et al.* (2014) analyzed chitosan derived from chitin isolated from *Fenneropenaeus indicus* for its physiochemical parameters, antibacterial and antifungal activity. Their data showed that the antimicrobial properties of chitosan were done at $120 \mu\text{gml}^{-1}$ and showed 12 mm inhibition zones toward both *S. aureus* and *Candida albicans*. Goy *et al.* (2016) evaluated the effect of commercial chitosan against *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) as reference bacteria. Their results, from turbidity reduction measurements, confirmed the antibacterial properties of chitosan.

On the other hand, several studies detected the bactericidal effectiveness of chitosan on Gram-positive and Gram-negative bacteria (Jeon *et al.*, 2001; No *et al.*, 2002; Coma *et al.*, 2003; Dutta *et al.*, 2009). These authors have stated that chitosan generally have stronger effects for Gram-positive bacteria (e.g. *Listeria monocytogenes*, *B. megaterium*, *B. cereus*, *S. aureus*, *Lactobacillus plantarum*, *L. brevis*, *L. bulgaris*, etc.) than for Gram-negative bacteria (e.g. *E. coli*, *Pseudomonas fluorescens*, *Salmonella typhimurium*, *V. parahaemolyticus*, etc.). The effectiveness of chitosan on gram-positive or gram-negative bacteria is however, somewhat controversial. One of the suggested hypothesis for this effect is declared by Raafet *et al.* (2008) who stated that, this difference in sensitivity is largely ascribed to the different structure of Gram positive and Gram negative bacteria cell envelopes, as a possible mechanism of action for chitosan antimicrobial activity is due to binding to

teichoic acids present in the cell wall of Gram positive bacteria, coupled with membrane lipids extraction which trigger a series of event that result in bacterial cell death eventually.

As bacteria, the chitosan activity against fungus is assumed to be fungistatic rather than fungicidal (Assis, 2008). Generally, chitosan has been reported as being very effective in inhibiting spore germination, germ tube elongation and radial growth (El Ghaouth *et al.*, 1992). Inhibition rate against plant fungus such as *Phomopsis asparagi*, *Cucumernum owen*, *Rhizoctonia solani* and *Fusarium oxysporum* have been known to occur with low chitosan concentration (20-150 mg l⁻¹) (Zhang *et al.*, 2003). However, most of the studies have been done on yeasts and moulds associated with food spoilage and plant diseases.

Table 4: Antimicrobial activity (mm) of extracted chitin and chitosan compared to commercial chitin and chitosan, values are mean of three independent experiments.

Microbe	Chitin		Chitosan	
	Extracted	Commercial	Extracted	Commercial
<i>E. coli</i> ATCC 8739	20	20	14	13
<i>E. faecalis</i> ATCC 29212	21	20	12	12
<i>S. aureus</i> ATCC 25923	23	22	16	15
<i>B. subtilis</i> ATCC 6633	22	22	16	16
<i>C. albicans</i> ATCC 10231	22	22	15	15

Indeed, discrepancies in the obtained results is recorded in many studies. This variation is expected to be due to both intrinsic and extrinsic factors. For instance, degree of deacetylation, viscosity, concentration, test microorganism, pH and temperature are all associated with the chitosan activity under *in vitro* conditions (Raafat and Sahl, 2009). For example, Malinowska-Pañczyk *et al.* (2015) estimated the antimicrobial properties of chitosan solutions and films against selected bacteria and the effect of chitosan incorporation into gelatin films. They found that the bactericidal effect of chitosan solutions increased with time and temperature of sample incubation. The degree of deacetylation has a major effect in the antimicrobial activity as it is determinant in the charge development and solubility of chitosan and increasing the degree of deacetylation will increase the free amino groups in the produced chitosan which leads to higher antimicrobial activity, as the -NH₂, -OH groups are considered the main reactive site in chitosan.

CONCLUSION

In this study, we extracted and characterized chitin and chitosan from *M. japonicus* shell. The current study supports the following points:

- 1- Shrimp shell waste (*M. japonicus*) can be used as a promising substrate for the chitin production using standard methods.
- 2- Deacetylation is an effective process to produce chitosan from the extracted chitin.
- 3- The chitin and chitosan exhibited considerable antimicrobial activities against pathogenic microorganisms compared to commercial chitin and chitosan.

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ARABIC SUMMARY

دراسة مقارنة على النشاط المضاد للميكروبات للكيتين والكيروزان التجاريين والمستخلصين من قشور الـ *Marsupenaes japonicas*

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تم إستخلاص الكيتين من قشور جمبرى *Marsupenaes japonicus* بنسبة ٢٧.٧% من الوزن الكلى عن طريق ثلاث خطوات، التخلص من البروتين والتخلص من اللون والتخلص من الاملاح. ثم تم الحصول على الكيروزان عن طريق نزع مجموعة الأسيتيل من الكيتين المنتج بنسبة ٥٥.١%. تم أيضا توصيف المركبات المنتجة عن طريق الفحص بواسطة المجهر الإلكتروني الماسح وتحليل FTIR والذي اظهر العديد من حزم الامتصاص فى المدى ٤٠٠-٤٠٠٠ سم^{-١}. وبالمقارنة أظهرت هذه الحزم تشابها مع تحليل الكيتين والكيروزان التجاريين، وأكدت على التخلص الجزئي من مجموعة الأسيتيل فى مركب الكيروزان المنتج. بالإضافة الى ذلك تم إختبار النشاط المضاد للميكروبات لكل من الكيتين والكيروزان المنتجين والتجاريين ضد مجموعة من البكتيريا المسببة للأمراض الموجبة والسالبة لصبغة الجرام والفطريات بإستعمال إختبار الإلتشار بالأجار. وقد أثبتت النتائج عدم وجود فرق معنوي $p < 0.05$ فى النشاط المضاد للميكروبات بين الكيتين والكيروزان المنتجين والتجاريين ضد مسببات الأمراض المستخدمة فى الإختبار، حيث سجلت المركبات المستخلصة مناطق تثبيط بقطر يتراوح من ١٢ الى ٢٣ مم، بينما سجلت المركبات التجارية مناطق تثبيط بقطر يتراوح من ١٢- ٢٢ مم، وكانت *Staphylococcus aureus* ATCC 25923 الأكثر تأثرا بقطر ٢٣ مم بواسطة الكيتين المنتج، فيما كانت *Enterococcus faecalis* ATCC 29212 الأقل تأثرا، حيث سجلت منطقة تثبيط بقطر ١٢ مم فقط بواسطة الكيروزان المنتج والتجاري.