Original Article

Structure and Antibacterial Activity of Chitosan from the American Cockroach, the German Cockroach and the Mealworm Beetle

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Abstract

Background: Owing to chitosan properties such as biocompatibility and antimicrobial activities, and several applications in biomedical field, some physicochemical and anti-bacterial properties, and the level of chitosan from three species of American cockroach, *Periplaneta americana* (Dictyoptera: Blattidae), the German cockroach, *Blattella germanica* (Dictyoptera: Ectobiidae) and the Mealworm beetle, *Tenebrio molitor* (Coleoptera: Tenebrionidae) were investigated.

Methods: The cuticle of adults derived from specimens was dried and grounded. The powders were demineralized as well as deproteinized after deacetylation via NaOH. At last, the chitosan yields from insects were studied for anti-bacterial activity on Gram-positive bacteria (*Proteus mirabilis*, *Klebsiella pneumoniae*), and Gram-negative bacteria (*Enterococcus faecalis* and *Staphylococcus epidermidis*). The Fourier transform infrared spectroscopy (FTIR) was used to analyze the chitosan composition.

Results: The chitosan ratios of the American and German cockroaches and the mealworm beetle were 5.80, 2.95, and 1.70% per 3 g of the dried bodies respectively. The chitin DD's for the American cockroach, the German cockroach and the mealworm beetle were 36.8%, 31.5% and 27.3%, respectively. The bactericidal activity of chitosan obtained from the American cockroach at a concentration of 1% had the greatest effect on *P. mirabilis* compared to other concentrations, while chitosan obtained from the German cockroach at a concentration of 0.01% had the greatest effect on *K. pneumoniae* compared to other concentrations.

Conclusion: According to the results, the anti-bacterial influence of the chitosan is based upon the insect species and chitosan concentration. Probably, the variation relates to the changes in the chitin structure among the three insect species.

Keywords: Chitosan; Cockroaches; *Tenebrio*; Anti-bacterial

Introduction

Chitosan, the chitin deacetylated derivatives, is a polysaccharide with a fibrous structure enormously detected in animals such as crustacean and insect exoskeletons (1). Chitin and chitosan is widely attended as a result of their useful biological characteristics like biodegradability, biocompatibility, non-antigenicity, and non-toxicity (2). Its exceptionally biological characteristics (anti-microbial, anti-bacterial, coagulating activities, bio-adhesivity, and wound healing capacity) caused it to be used in cosmetics, medicine and pharmacy, agriculture, food industry, and wastewater treatment (3, 4). Currently, more attention has been paid to the producing of chitin and chitosan from insect sources. Firstly, insects have extensive biodiversity and show 95% of the animal group (5). Thus, they, as a natural source, have a great capacity to produce chitin and chitosan. In addition, the inorganic content of insect cuticles is less than that of crustacean shells, causing their demineralization to be extremely appropriate (6).

The physicochemical properties of chitosan

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have been examined in several investigations employing element analysis, differential scanning calorimetry (DSC), scanning electron microscopy (SEM), and X-ray diffraction patterns (7, 8). Chitosan's activity depends on the yields, molecular weight (MW), level of deacetylation (DD), and amino groups (NH_2) presence (9). According to research by currently, different researches have investigated the powerful anti-microbial influence of chitosan on various microorganisms, from bacteria (10) to fungi (11), parasites (12, 13), and yeasts (14) in tests including in vivo or in vitro interactions with various chitosan (solutions, films, and composites). Therefore, studies on chitosan and its antimicrobial ability have recently been important (15, 16). Molecular weight (MW) plus the degree of deacetylation (DD) become essential to the chitosan activity, and also on some experimental conditions, such as temperature and pH (17). Basseri et al. (18) showed the degree of DD of chitin was 31% and 32.1% for the German cockroach nymphs and adults, respectively, and 39.2% and 37.3% for the American cockroach nymphs and adults. In all cases, the weight of chitosan's produced was almost half that of chitins. They tested solutions of chitosan at the concentration of 10mg/ml on three different bacteria: *Escherichia coli* and *Pseudomonas aeruginosa*, as Gram-negative, and *Staphylococcus aureus*, as Gram-positive species. Also, Chung et al. have shown the disruption of cell structure of *E. coli* and *S. aureus* due to the binding of chitosan to microbial enzymes and nucleotides (18). However, chitosan molecules show different influences on various microorganisms (19, 20). It has been suggested that the bactericidal mechanism of chitosan depends on the existence of a positively charged molecule (NH^{3+} sites) interacting with the negatively charged membrane of a microbial cell, causing the ammonia group to bind as a protonated molecule to the negative residues by electrostatic forces (5).

Chitosan has been effectively used in various fields such as environmental applications especially in water, paper, and textile treatment for antimicrobial activity, biomedical applications such as tissue engineering, wound healing, obesity treatment, preventing vascular diseases, and food industrial applications such as food packaging film, nanocapsule and nanoparticles (1). Despite different investigations on the antimicrobial influences of chitosan (16, 21, 22), no consensus has yet been reached. Therefore, additional investigations are needed.

Considering these useful functional properties of chitosan, this study focused on chitosan from edible insect, which has not been investigated sufficiently. To investigate functional properties of edible insect chitosan from the mealworm beetle (*Tenebrio molitor*), and to find other possible uses as a new biomaterial, compared to insects of hygiene pest such as the American cockroach (*Periplaneta americana*) and the German cockroach (*Blattella germanica*). This study aimed to extract the chitosan from the American cockroach, the German cockroach and the mealworm beetle, to compare their structural homology, and then to measure the antimicrobial activities of the resulting extracted chitosan's against four strains of bacteria: *Proteus mirabilis, Klebsiella pneumoniae, Enterococcus faecalis* and *Staphylococcus epidermidis*. Selection of these bacteria was due to their role as nosocomial infections and human pathogenicity. According to previous studies (23), these bacteria exist symbiotically in the body of the studied insects in laboratory and environmental conditions.

Materials and Methods

Sample collection

The adult cockroaches were provided from the Laboratory of Medical Entomology, Tehran University of Medical science (TUMS), and the mealworm beetle from the Laboratory of Entomology, University of Qom. The insects were kept in an insectary at 25±2 °C with 12h: 12h light–dark cycle. Their food was dried bread, date, and water. They hungered

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for 48h so that the gut contents emptied. Next, they were killed through freezing at -20 ℃, and the body was washed with water. They were dried through heating at 50 °C for 24h. Next, the body was mechanically ground and filtered by a 20-mesh sieve (21). At last, 3g of powder of every sample was utilized to extract chitosan.

Extraction of Chitin and Chitosan

Chitosan was obtained from insect processing discards via a Chang - proposed method (24). To deproteinize, 3g of powder of species were distinctly treated with 1M HCl at 100 ℃ for 24h, filtered through a 20-mesh sieve, washed with distilled water, and treated with oxalic acid for 3h at ambient temperature with moderate stirring. Consequently, the demineralization process was performed through filtering the treated specimens by a 20-mesh sieve as well as washing them in distilled water. The process continued with adding 50ml of 1% sodium hypochlorite solution to each sample and locating at ambient temperature for 3h with moderate stirring to eliminate the color of the samples. Extracted chitins were filtered via a 20-mesh sieve, washed in distilled water, and dried overnight at 60 °C. The yields were treated via 50% NaOH at 100 °C by moderate stirring for 4h, then washed in distilled water and ethanol so that the acetyl group was eliminated from chitins. The procedure was repeated three times.

The chitosan was dried at room temperature and was put in a clean and dry container. The chitosan was dissolved in 1% acetic acid (Sigma-Aldrich, MI, USA) to obtain a starting concentration of 10mg/ml. One gram of chitosan was dissolved in 100mL of acetic acid solution by stirring for 3h (Hotplate Magnetic Stirrer) at 50 ℃. Hence, the chitosan used in the succeeding assays were dissolved in 1% acetic acid. Previous studies used 1% acetic acid despite its anti-bacterial activity (22, 25). Different chitosan concentrations (0.01, 0.1, and 1%) were prepared through dilution of 1% stock solution. Similar procedure was done to test the antibacterial activity of commercial chitosan, was purchased from Sigma-Aldrich (CAS-No: 9012-76-4, Chemie GmbH Eschenstrasse, Germany).

Fourier Transform Infrared (FTIR) analysis

To identify the composition of chitin and chitosan, and the degree of acetylation (DA), the analysis of specimens was performed via Fourier-transform infrared spectroscopy (FTIR) (Tensor 27, Bruker) at the Central Laboratory, University of Tehran (Tehran, Iran) at 4,000–500cm^{-1} with potassium bromide (KBr) pellets. Commercial chitin and chitosan obtained from Sigma Aldrich –were considered criteria. The wavelength range was 500–4,000cm^{-1} at a resolution of 4cm^{-1}. The absorbance of the peaks was compared with that of the reference peak at A1655/A3450 (26). Furthermore, the chitin deacetylation degree (DD) was evaluated. The findings of the obtained chitosan were compared to the commercial one. The deacetylation degree (DD) was found by the following equation (27):

$$DD\ (\%) = 100 - [(A_{1655} / A_{3450}) \times 100]/1.33$$

In which A1655= mean% absorbance before and after wavenumber 1655. A3450= mean % absorbance of wavenumber 3450.

Scanning Electron Microscopy (SEM)

Scanning electron microscope (SEM; Zeiss DSM 960A, Carl Zeiss, Oberkochen, Germany) was utilized to test the chitin surface morphology at the Central Laboratory, University of Tehran (Tehran, Iran). The samples of chitosan were ground, located on an adhesive tape as well as coated with a fine gold layer via sputter coater. The SEM was performed at 20.0kV.

Bacterial Strains

The bacterial strains, including *P. mirabilis* (ATCC 43071), *K. pneumoniae* (ATCC 1705), *E. faecalis* (ATCC 29212), and *S. epidermidis* (ATCC 12228) were provided with the Industrial Research Organization of Iran.

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Each bacterium was inoculated into an Erlenmeyer flask consisting of 100ml of sterile nutrient broth (peptone 1%, beef extract 0.5%, NaCl 0.5%, pH 6) and incubated at 37 °C for 24h. Sterile Mueller Hinton Agar (MHA, Himedia) medium was arranged in sterile Petri dishes, incubated at 37 °C for 24h as well as utilized to test antibacterial activity.

Anti-bacterial Assays

The disc diffusion method was employed in this study for antimicrobial assay (28). Firstly, 20µL of freshly bacterial cultures of *P. mirabilis*, *K. pneumoniae*, *E. faecalis*, and *S. epidermidis*, equal to 0.5 McFarland prepared, were then spread uniformly onto Mueller–Hinton agar plates. Chitosan sample discs-prepared by impregnating 50µL of chitosan solution on sterile filter paper discs (6mm diameter), were placed on the agar plates. The plates were then incubated at 37 °C for 24h using an incubator (IN55, Memmert, Germany). The presence of inhibition zones was measured around each disc in millimeters (mm) by a metric ruler and was considered as evidence of antimicrobial activity. The experiments for each test organism were carried out in triplicate. For each plate, filter-paper discs soaked of acetic acid and solution of commercial chitosan were used at same concentrations as positive controls, while distilled water as a negative control.

Statistical analysis

All steps were done in triplicate. SPSS (version 25, Ins. USA) was utilized to analyze data. Results were expressed as mean ± standard error of growth inhibition zones diameters obtained with extracted chitosan, which amount was adequate for repetitions. Statistical differences of diameter of growth inhibition zones between the insects-derived chitosan, the bacterium type, and concentration of extracted chitosan were determined by analysis of variance. The LSD test was used to determine the difference among means at the level of 0.05.

Results

Chitin and Chitosan extraction

Chitin and chitosan obtained from 3g of dried insect powder differed in terms of insect species. Comparably, the chitosan yield amount was nearly half of the chitin one (Fig. 1). The chitosan ratios per 3g of the dried body in the American cockroach, the German cockroach and the mealworm beetle were 5.8, 2.95, and 1.7%, respectively. The degrees of chitin deacetylation (DD) of all selected samples are calculated by FTIR analysis and shown in Table 2. The chitin deacetylation degree for the American cockroach, the German cockroach and the mealworm beetle was 36.8%, 31.5%, and 27.3 %, respectively; illustrating that the extracted chitosan of German cockroach is more deacetylated than the other chitosan (Fig. 1).

Scanning electron microscopy of extracted chitosan

Under electron microscopic examination, the extracted chitosan's of the American cockroach, the German cockroach and the mealworm beetle showed similar microfibrillar structure. However, commercial chitosan did not exhibit an apparent microfibrillar structure. The extracted chitosan's of the American cockroach and the German cockroach exhibited rough and thick surface morphology more than the mealworm beetle (Fig. 2). At the SEM photographs, chitin of the American and German cockroaches (Fig. 2a, b) markedly arranged in a microfibrillar crystalline structure was obvious compared to chitin of the mealworm beetle (Fig. 2c).

Fourier transform infrared spectroscopy Analysis

Based on the FTIR graph, the molecules of chitin and chitosan of three groups consist of the same stretching, bending vibration bands with various infrared spectrum graphs (Fig. 3), indicating decreased peaks because of the absorbing, which shows a loss of acetyl group and chitin deacetylation.

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The absorption bands of spectra at 1560–1630cm^{-1} (amid I stretching in C=O) and 1370–1400cm^{-1} (NH2 binding) determined two prominent amide bands. The absorption band at 1010–1030cm^{-1} shows C–O–C stretching vibrations existing in chitosan molecules (Fig. 3).

The existence of a chitosan absorption band of the C-H stretching, bending vibration along with the C–O–C stretching vibrations, particularly in the specimen from the mealworm beetle, are presented in Fig. 3. The absorption bands are created via the stretching, C-H bending vibrations existing in their chitosan molecules. The broad and wide wavelength on commercial chitosan from the American cockroach, the German cockroach and the mealworm beetle (with a peak of 3249.1, 3260.9, 3250.2, and 3210.2cm^{-1}, respectively) show the existence of hydroxyl group (O-H) in total samples, while sharp peaks at 2900.47cm^{-1}, 2919.36cm^{-1}, 2917.49cm^{-1} and 2910.12cm^{-1} of the commercial plus obtained chitosan from the American cockroach, the German cockroach and the mealworm beetle, respectively, show strong existence of alkanes in the specimens. This supports the chitosan correct chemical structure mainly composed of a C-C single bond.

Anti-bacterial Activities Analysis

Table 2 shows the antibacterial activities of chitosan obtained from the insects. The findings indicate the effect of the extracted chitosan on Gram-positive and Gram-negative bacteria, including *P. mirabilis, K. pneumoniae*, *E. faecalis* and *S. epidermidis*. Also, the zone of inhibition for different concentrations of chitosan against the tested bacteria is presented in Table 2. The extracted chitosan shows different levels of antibacterial activity on Gram-positive bacteria versus Gram-negative bacteria. The results showed that the antibacterial activity of chitosan obtained from the American cockroach with a concentration of 1% had the most significant effect on *P. mirabilis* (Gram-negative), when was compared to standard chitosan (p= 0.000). The antibacterial activity of chitosan obtained from the American cockroach at a concentration of 1% had the most significant effect on *P. mirabilis* compared to other concentrations of chitosan extracted from the American cockroach (p= 0.000). The antibacterial activity of chitosan obtained from the American cockroach with a concentration of 0.1% had the most significant effect on *S. epidermidis* (Gram-positive), when was compared to standard chitosan (p= 0.003). Also, the results showed that the antibacterial activity of chitosan obtained from the American cockroach with a concentration of 1% and 0.01%, almost with the same inhibition zone, had the most significant effect on *K. pneumoniae* (Gram-negative) compared to the concentration of 0.1% (p= 0.000). Overall, the antibacterial activity of chitosan obtained from the American cockroach at a concentration of 1% compared to other concentrations had the most significant effect on *P. mirabilis* (Gram-negative) than other bacteria (p= 0.000) (Table 2).

The results showed that the antibacterial activity of chitosan obtained from the German cockroach with a concentration of 1 and 0.1% had the most significant effect on *P. mirabilis* (Gram-negative), when was compared to standard chitosan (p= 0.000). The antibacterial activity of chitosan obtained from the German cockroach at a concentration of 0.01% had the most significant effect on *K. pneumoniae* (Gram-negative) compared to standard chitosan (p= 0.000). Also, the results showed that the antibacterial activity of chitosan obtained from the German cockroach with a concentration of 0.01% had the most significant effect on *S. epidermidis* (Gram-positive) compared to other concentrations (p= 0.002). Overall, the antibacterial activity of chitosan obtained from German cockroach at a concentration of 0.01% compared to other concentrations had the most significant effect on *K. pneumoniae* (Gram-negative) than other bacteria (p= 0.000) (Table 2).

However, the antibacterial activity of chitosan obtained from the mealworm beetle had

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the least effect compared to others, so that only 1% concentration had the most significant effect on *P. mirabilis* (Gram-negative), when was compared to standard chitosan (p= 0.003) (Table 2).

The results showed that the antibacterial activity of chitosan obtained from the American cockroach at a concentration of 1% had the greatest effect on *P. mirabilis* (Gram-negative) compared to other bacteria in different concentrations of chitosan obtained from insects. Also, the results showed that the antibacterial activity of chitosan obtained from the German cockroach with a concentration of 0.01% had significant effect on both Gram-positive and Gram-negative bacteria. In addition, the results showed that insect-derived chitosan has a great inhibitory effect on Gram-negative compared to Gram-positive bacteria (Table 3, Fig. 4).

The mean± SEM of diameter of growth inhibition zone (mm) were measured and recorded as recommended by World Health Organization 2003 (49). In addition, all significant values of antibacterial activity between chitosan of cockroach and beetles compared to standard chitosan are given in brackets. Here, superscript [a] stands for the best of growth inhibition zone among bacteria types in the same concentration from the same insect's extracted chitosan, and superscript [b] stands for the best of growth inhibition zone among the different concentrations of the extracted chitosan in the same insect that affected on the same bacteria, and superscript [c] stands for the best of antibacterial activity among the same bacteria and same concentration of the extracted chitosan from different insects. The rest of the cases that are not mentioned p value, were not significant compared to standard chitosan. The experiment was conducted in triplicate.

Table 1. The degree of deacetylation of the insect's chitin using infrared spectra analysis at 4,000–500cm^{-1}

Insect species	A1655	A3450	DD
German cockroach	0.153	0.182	36.8
American cockroach	0.126	0.149	31.5
Mealworm beetle	0.175	0.181	27.3

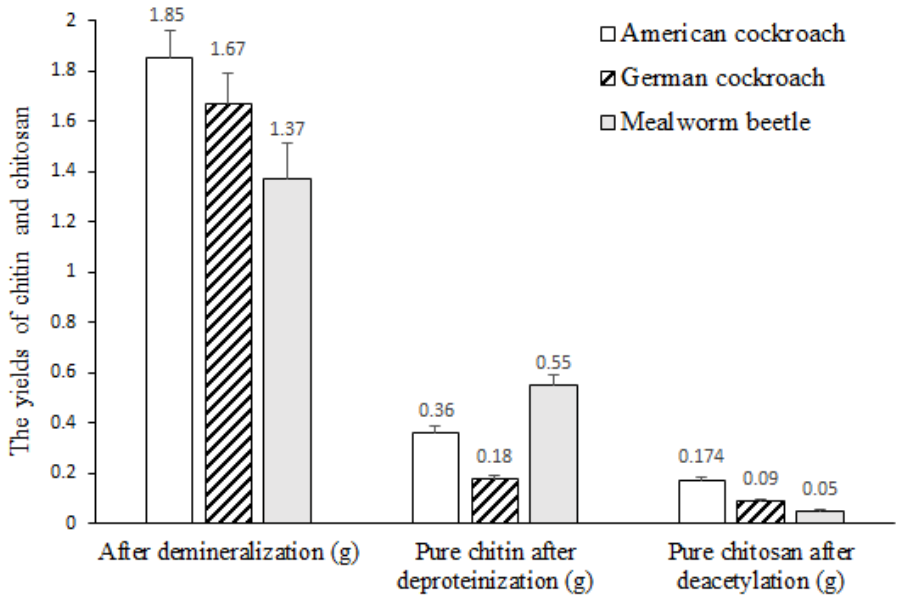

Fig. 1. The yields of chitin and chitosan obtained from 3-g insects' powder after extraction process

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Table 2. Anti-bacterial activities of yield chitosan extracted from adults of insects measured based on the diameter of growth inhibition zone (mm)

Chitosan extracted	Concentration of chitosan (%)	Zones of growth inhibition			
		(G-) Proteus mirabilis (p value)	*(G-) Klebsiella pneumoniae* (p value)	*(G+) Enterococcus faecalis* (p value)	*(G+) Staphylococcus epidermidis* (p value)
American cockroach	1	12.2±0.3 (0.000) [a, b, c]	10.2±0.2 (0.000) [b]	6.2±0.1	8.2±0.2 (0.000)
	0.1	8.2±0.2	9.2±0.1	7.2±0.2	7.2±0.2 (0.003) [a]
	0.01	10.2±0.2 (0.003)	10.2±0.2 (0.000) [a]	8.2±0.1	8.2±0.2
German cockroach	1	11.2±0.3 (0.000) [a]	9.2±0.2 (0.008)	8±0.3	8.2±0.2 (0.000)
	0.1	11.2±0.1 (0.000) [a]	10.2±0.2	7.2±0.1	7.2±0.2 (0.003)
	0.01	8.2±0.1	11.2±0.3 (0.000) [a, b, c]	8.2±0.2	9.2±0.2 (0.002) [b, c]
Mealworm beetle	1	9.2±0.2 (0.003) [a, b]	8.2±0.1	7.2±0.2	6.2±0.2
	0.1	9.2±0.2	9.2±0.2	6.2±0.1	6.2±0.2
	0.01	8.2±0.2	9.2±0.2	6.2±0.2	8.2±0.1
Standard (Commercial chitosan)	1	8.2±0.2	8.2±0.1	8.2±0.2	6.2±0.2
	0.1	9.2±0.3	10.2±0.2	8.2±0.1	6.2±0.2
	0.01	9.2±0.2	9±0.0	8.2±0.2	8±0.3

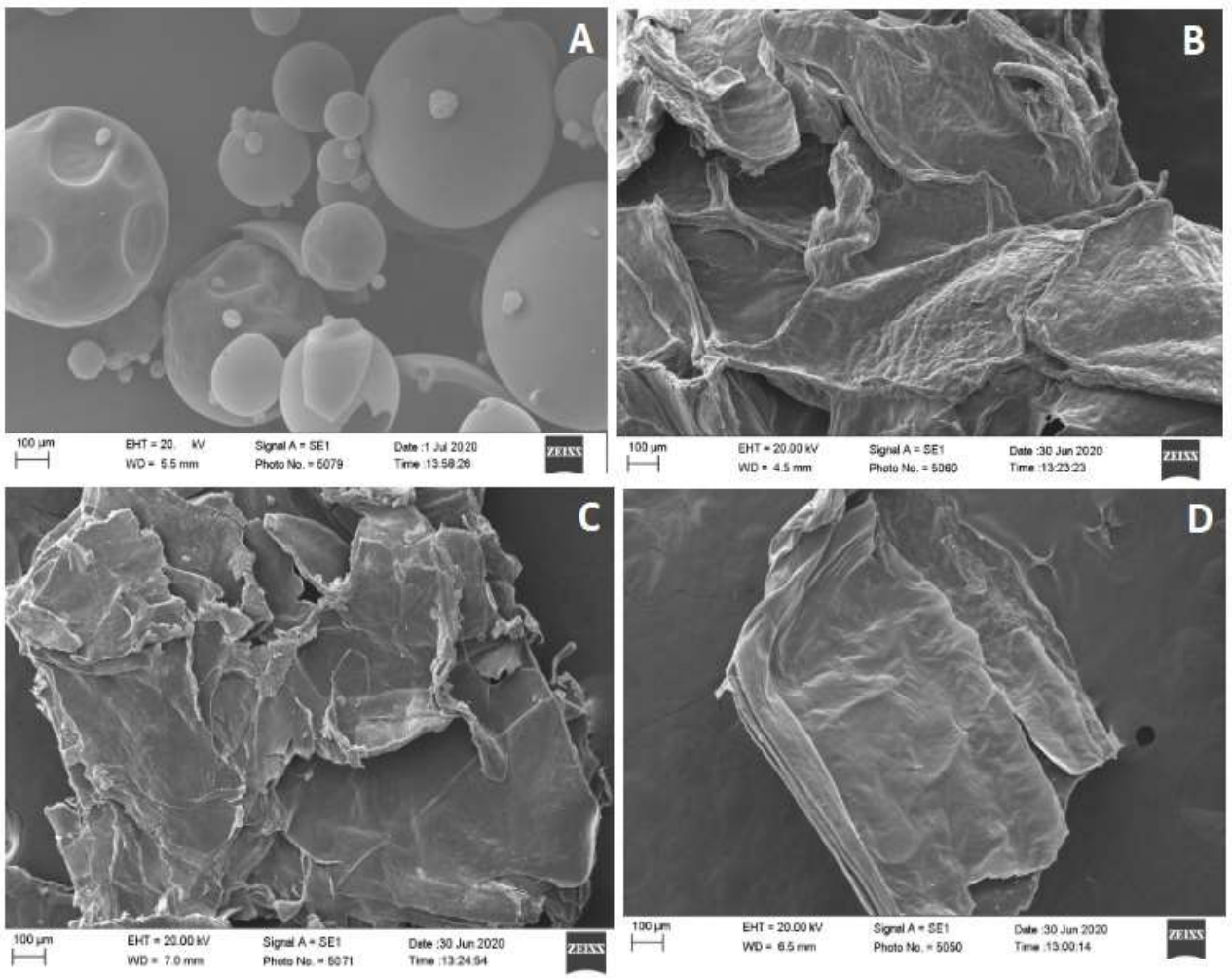

Fig. 2. SEM micrographs of chitosan extracted from adults of insects: A) Standard (Commercial chitosan), B) American cockroach, C) German cockroach, D) Mealworm beetle

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Table 3. The best of anti-bacterial activity yields the concentration of extracted chitosan from adults of insects on the Gram-positive and Gram-negative bacteria

Chitosan source	Gram-negative (concentration%)	Gram-positive (concentration%)
American cockroach	12.2±0.3 (1)	8.2±0.2 (1)
German cockroach	11.2±0.3 (0.01)	9.2±0.2 (0.01)
Mealworm beetle	9.2±0.2 (1)	8.2±0.1 (0.01)
Standard (Commercial chitosan)	10.2±0.2 (0.1)	8.2±0.1 (0.1)

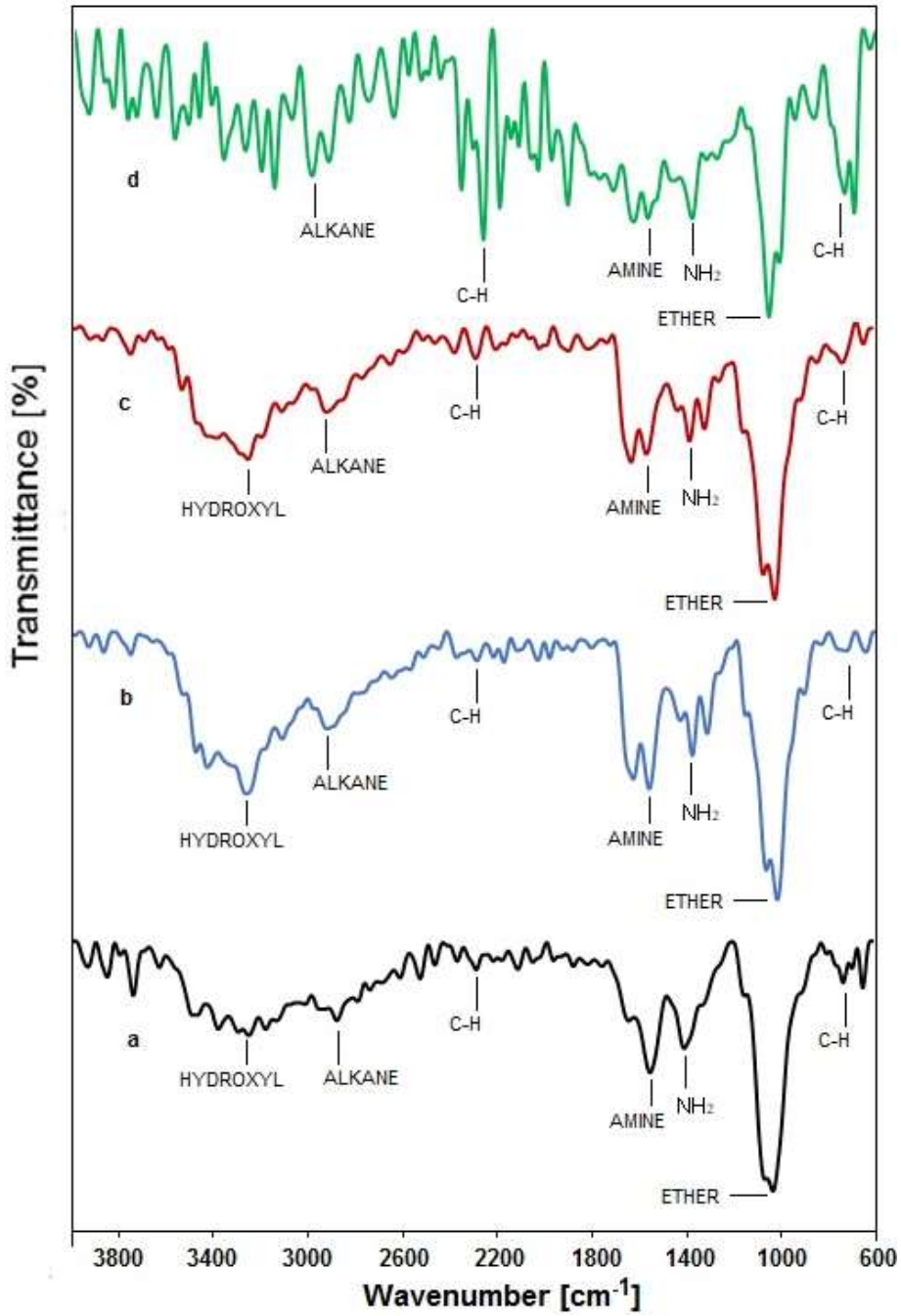

Fig. 3. Infrared spectra of Commercial and Extracted Chitosan. a) Standard (Commercial chitosan), b) American cockroach, c) German cockroach, d) Mealworm beetle

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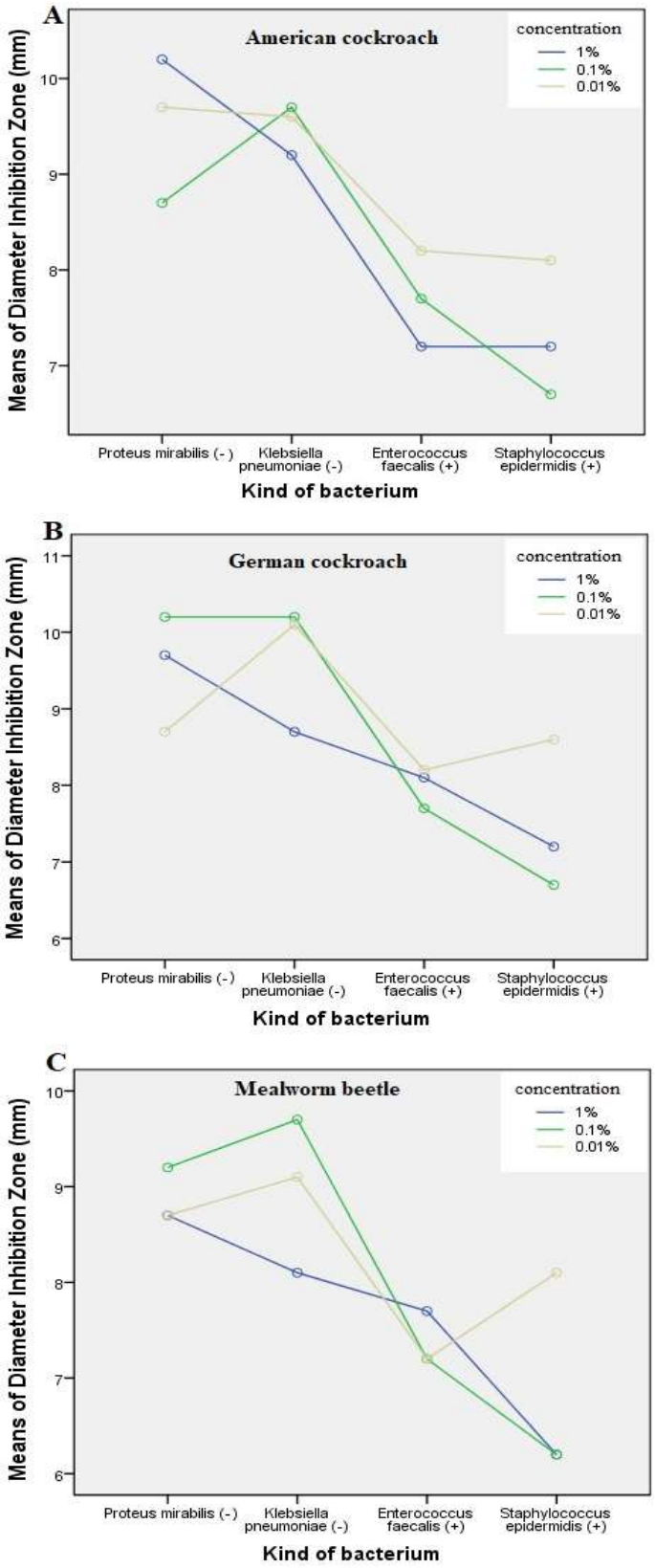

Fig. 4. Comparison of the growth inhibitory effect of different concentrations of extracted chitosan from insects on Gram-positive and Gram-negative bacteria. A) American cockroach, B) German cockroach, C) Mealworm beetle

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Discussion

In this research, chitin and chitosan of the American cockroach, the German cockroach and the mealworm beetle were prepared and partly determined. Then, the DD was measured. Afterward, bactericidal activities of the chitosan yield were studied. In the investigation, the antimicrobial activity of the obtained chitosan was varied among these insects. Furthermore, the degree of polymerization and crystallinity of chitin and chitosan of the American cockroach, the German cockroach and the mealworm beetle were various.

According to several previous studies on chitin yield from other insects depending on growth stage, the chitin yield ranged from 5.3 % to 36.6% as follows: seven Orthoptera species contained 5.3–8.9% (29), *Holotrichia parallela* contained 15% (30), *Ranatra linearis* contained 15–16% (31), and cicada contained 36.6 % (32). In this study, the chitin yield from cockroaches was higher than from the mealworm beetle. Physicochemical characteristics, rheological characteristics as well as surface morphology of chitosan of cicada slough, silkworm chrysalis, mealworm, or grasshopper were compared to shrimp shell chitosan. According to findings, the chitosan activities of insects are completely distinct from shrimp chitosan (5). Furthermore, the chitosan anti-microbial activities are based upon DD (33). In this case, the chitosan of insects is indicated to be much viscous compared with shrimp shell chitosan having a high deacetylation degree. Typically, part of chitosan bacteriostatic and bactericidal activity is based upon viscosity. Low viscosity is very efficient (5, 34). Chitosan DD of three insects was varied due to variation of chitosan antibacterial characteristics. The main element of the insect integument has usually been known to be an efficient substitute source having organic materials like chitin, especially the cuticle having decreased inorganic materials (35). Compared to commercial chitosan's yield from shrimp (26), the chitosan yields of the cockroaches were high within demineralization and deacetylation processes. While insects including cockroaches may be abundant and accessible chitin sources of chitin and chitosan, rearing them is a limiting factor for industrialization. The cockroach species-derived chitins illustrated the same physiological characteristics. They seem to be appropriate for chitosan production. While the chitin-chitosan yield from the American cockroach increases, the chitin /chitosan DD was somewhat high in the German cockroach-derived specimens. The variation of molecular weights of chitosan obtained from both groups leads to these findings. The finding is consistent with previous findings (21, 36).

According to the literature, surface morphology is one of the most important properties that determines the efficient use of chitin and its derivatives (4). The best usage area for chitin can be determined according to its surface morphology. The number of pores in the chitin surface increased the chitin's ability to absorb metal ions, while the chitin that has a fibrillar surface morphology can be used in textiles (29). In addition, a porous structure means the chitin can be a useful agent for tissue engineering (4). It can be seen from previous studies that the surface morphologies of chitin and its derivatives obtained from crab, krill, insects and fungi are quite different (29). In this experiment, the extracted chitosan's of the American cockroach and German cockroaches exhibited rough and thick surface morphology with a microfibrillar crystalline structure, which was like the findings of previous studies (26, 30). At the SEM photographs, chitin of the American and German cockroaches markedly arranged in a microfibrillar crystalline structure was obvious compared to chitin of the mealworm beetle. Similar results can be seen in the SEM photographs of the beetle chitin from cicada sloughs (32). The FTIR results suggest that there was a similarity between the chemical composition and the bonding types of chitosan in the extracted chitosan's and commercial chitosan. The present findings showed

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that the peaks at around 1560–1630cm^{-1} and 1370–1400cm^{-1}, which correspond to (C= O) in the NHCOCH3 group (amide I band) and (NH2) in the NHCOCH3 group (amide II band), respectively, were characteristic of chitosan. These present findings are consistent with previous reported (21, 30). The peaks displayed at around 1010-1030cm^{-1} were attributed to the β (1–4) glycosidic bond in the polysaccharide unit and the stretching vibrations of C-O-C in the glucose ring. These findings are similar to previous reports (21, 30, 36). Additional broad absorption bands observed at 2900–3250cm^{-1} were attributed to symmetric stretching vibrations of the O-H and alkane caused by the strong intermolecular hydrogen bonding of chitosan polysaccharides. These present findings are consistent with previous reports (21, 30).

In this research, it was determined that the chitosan of groups inhibited the growth of Gram-positive and Gram-negative bacteria. Generally, many elements can have influenced the bactericidal activity strength of chitosan, such as the molecular weight, deacetylation degree, chitosan concentration in solution, or pH of medium culture (37). Its anti-bacterial effect has been said to be highly dependent upon its molecular weight in lieu of the DD (38). Reduced molecular weight indicated great inhibiting influence on Gram-positive, Gram-negative bacteria and the yeast (39). However, chitosan anti-microbial characteristics are based upon various factors and may cause different results mentioned by several authors. Thus, the bactericidal activity of chitosan is slightly debatable. Chitosan is said to have high bactericidal activity on Gram-positive bacteria in comparison to Gram-negative ones (15, 40). Conversely, some authors mentioned, owing to the hydrophilicity of chitosan, Gram-negative bacteria are very susceptible to chitosan compared to Gram-positive ones (37).

In the current research, among the examined bacteria, Gram-negative ones became exceedingly susceptible to the cockroach chitosan. It is assigned to a high deacetylation degree of chitosan. Some authors have noted chitosan influence on Gram-positive bacteria is greater in comparison to Gram-negative ones (41, 42). The bacterial influence on Gram-positive along with Gram-negative bacteria is somewhat debatable. In contrast, hydrophilicity in Gram-negative bacteria has been illustrated to considerably increased in comparison to in Gram-positive bacteria, causing them to be susceptible to chitosan (43). The results are proven through many *in vitro* tests where Gram-negative bacteria are significantly susceptible to chitosan, indicating enhanced morphological changes in treatment in comparison with Gram-positives (44-46). Chitosan from the mealworm beetle showed slight inhibition zones against *Bacillus cereus, Listeria monocytogenes* and *E. coli*, and also slight inhibition zone against *S. aureus* in antimicrobial activity test (47). This finding is consistent with our findings. Also, this experimental study showed for the first time that chitosan from the mealworm beetle has antimicrobial activity against pathogenic bacteria such as on Gram-positive bacteria (*P. mirabilis*, *K. pneumoniae*) and Gram-negative bacteria (*E. faecalis*, *S. epidermidis*).

A crucial factor is establishing the adsorbed chitosan level is the charge density on the cell surface (48). The chitosan binding to microbial DNA is the additional suggested mechanism. This results in suppressing the mRNA plus protein synthesis by the chitosan entry to the nuclei of the microorganisms (45). Another mechanism is the metal chelation, spore component inhibition as well as binding to essential nutrients for microbial growth (15). The Gram-negative bacteria cell wall is very complicated but thinner than that of Gram-positive ones. It includes a semi-permeable outer membrane locating on a peptidoglycan layer suppressing the antibiotic penetration into the cell (22). It is an asymmetric lipid bilayer consisting of lipopolysaccharide (LPS). Interaction between chitosan and Gram-negative bacteria via electrostatically interacting with the negatively charged LPS changing permeability (22).

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Conclusions

Based on the results, we found the amount of chitosan yield and the degree of deacetylation depended on the species of insects. The anti-bacterial influence of the chitosan is based upon the cockroach species. The chitosan obtained from cockroaches, especially the American cockroach, showed a high impact of inhibition on the Gram-negative bacteria. The variation likely is because of variations in the chitin structure among the three insect species.

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Ethical consideration

The ethics committee of Ardabil University of Medical Sciences has proved the study (IR.ARUMS.REC.1398.617).

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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