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Research Article

COMPARATIVE STUDY OF PHYSIOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF ADULT COCKROACHES-EXTRACTED CHITOSAN

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ABSTRACT. Drug resistance, caused by the overuse of antimicrobial drugs, has necessitated the development of new strategies for treatment. Insect species, the largest in the animal kingdom, can serve as a suitable source for the production of chitosan, which is an important immune system stimulator. We investigated the physicochemical and antimicrobial properties of chitosan extracted from adult cockroaches of *Blattella germanica* (Dictyoptera: Ectobiidae) and *Periplaneta americana* (Dictyoptera: Blattidae). The cuticle of the adult insects was dried and grounded. Subsequently, samples were deproteinized, demineralized, and then deacetylated using NaOH. The FTIR spectra were employed to evaluate the functional groups of chitosan. Antimicrobial action was investigated against Gram-positive bacteria (*Staphylococcus epidermidis* and *Enterococcus faecalis*) and Gram-negative bacteria (*Klebsiella pneumoniae* and *Proteus mirabilis*). The results indicated that the chitosan ratios in American and German cockroaches were 5.80 and 2.95%, respectively, for every 3 g of powder obtained from each sample. Additionally, the deacetylation degree (DD) of chitin in German and American samples was 31.5% and 36.8%, respectively. At a 1% concentration, German cockroach chitosan exhibited significant bactericidal activity against *K. pneumoniae* compared to other concentrations, while the 1% concentration of American cockroach chitosan had the highest efficacy against *P. mirabilis* compared to other concentrations. This study demonstrates that the chitosan concentration and insect species have an impact on the bactericidal action of chitosan.

Keywords: *Cockroaches, chitosan, chitin, antimicrobial.*

INTRODUCTION

All over the world, the emergence of bacterial resistance can threaten advances made in healthcare, food production, and ultimately life expectancy. Considering the resistance of most bacterial species against on highly toxic antibacterial drugs, there is a need for innovative strategies in this field [1]. Since the habitat of most insects, including cockroaches, are in dirty areas, they seem to have developed efficacious ways to fight

against infectious agents [2]. A large amount of chitosan and deacetylated chitin derivatives are extracted in the form of a fibrous polysaccharide from animals such as the exoskeletons of crustaceans and insects, and these have been widely used in pharmaceutical excipients [3, 4].

The valuable biological features of chitin and chitosan, including biodegradability, biocompatibility, antimicrobial, antibacterial, coagulation activities, wound healing action, and bio-adhesion have led to their usage in diverse fields such as wastewater treatment, pharmacy, cosmetics, medicine, food industry, and agriculture [5, 6]. Insects, being the largest animal species with vast biodiversity, possess significant potential as a native source for chitosan and chitin production. Consequently, the extraction of chitin and chitosan from insect sources has garnered much attention [7]. Furthermore, the demineralization of insect cuticles is relatively straightforward due to lower mineral levels compared to crustacean shells [8]. Researchers have investigated the antimicrobial effects of different forms of chitosan (film, solution, and composite) on various microorganisms, including bacteria [9, 10], fungi [11], parasites [12], and yeasts [13], either *in vitro* or *in vivo* were investigated by many researchers. Indeed, chitosan and some of its properties, such as its antimicrobial activity have recently gained enormous attention [14].

Chitosan polymer exhibits various mechanisms to inhibit the growth of microbes that are yet to be fully understood. One of these mechanisms involves altering the membrane permeability of microbes through electrostatic interactions of its polycationic structure (-NH^{3+} sites) with the negatively charged cell membrane of microbes [7]. Despite numerous examinations on the antimicrobial effects of chitosan, no absolute agreement has been reached [15, 16], indicating the need for additional research. As the threat of antimicrobial resistance continues to rise, there is an increasing demand for the discovery of new antibiotics from various sources such as plants, fungi, and other organisms. The present study aimed to compare the levels of chitosan obtained from two insects (American cockroach and German cockroach). Additionally, the antibacterial features of the chitosan extracted from the two insects were investigated against Gram- positive bacteria (*Staphylococcus epidermidis* and *Enterococcus faecalis*) and Gram- negative bacteria (*Klebsiella pneumoniae* and *Proteus mirabilis*). Bacteria were chosen for this study due to their significant role in human pathogenesis and hospital infections. Previous studies [4, 17], have demonstrated the symbiotic presence of bacteria in the bodies of insects.

MATERIALS AND METHODS

Ethical Approval

The study was approved by the Institutional Review Board, and the approval (IR.ARUMS.REC.1398.617) for the research was granted by the ethical committee of Ardabil University of Medical Sciences.

Sample Collection

The adult insects obtained from the Medical Entomology Laboratory of Tehran University of Medical Sciences were housed in an insectary at 25±2°C with a light/dark cycle of 12/12 hours and were provided with a diet of dry bread, water, and dates. They were subjected to a 48-hour period of starvation to empty the contents of their gut. The

insects were then euthanized at -20°C. Subsequently, their bodies were rinsed with water, dried at 50°C for 24 hours, manually ground, and sieved through a 20-mesh sieve [15].

Extraction Method

The Chang-proposed method was employed for extracting chitosan from insects [18]. Three g of powder from each sample was used for deproteinization. Initially, cockroach's exoskeletons were decalcified for 24 h in 500 mL of IM HCl at 100 °C. Subsequently, the samples were incubated in 500 mL of 1.25 N NaOH at 95°C for 3 h to remove proteins. The deproteinized samples were filtered using a 20-mesh sieve, washed with water, and treated with oxalic acid (3 h) at room temperature. For decolorization, each sample was blended with 50 ml of NaClO (sodium hypochlorite solution, 1%, w/v) and gently stirred at room temperature for 3 h. The resulting chitins in this process were filtered with a 20-mesh sieve, rinsed with water, and dehumidified at 60°C (overnight). To eliminate the acetyl group from the obtained products (chitins), they were treated with NaOH (50%) for 4h at 100°C under mild stirring, then rinsed with distilled water and ethanol. This stage was repeated three times. After dehumidification at normal temperature, the chitosan was stored in a dirt-free and dry box. One g of extracted chitosan was dissolved in acetic acid (100 mL, 1% v/v) at 50°C for 3 h under shaking to prepare an initial concentration of 10 mg/ml. Different concentrations (0.01, 0.1, and 1%) of chitosan were prepared by diluting the 1% stock solution. The same procedure was followed to evaluate the antimicrobial effect of commercial (criterion) chitosan obtained from Sigma-Aldrich.

FTIR Analysis of Chitosan

Fourier-transform infrared spectroscopy (FTIR) was employed to characterize the characterization of chitin, chitosan, and the degree of acetylation (DA). FTIR spectra were recorded by mixing the samples with KBr, ranging between 4000 and 500 cm^{-1}. Commercial chitosan and chitin purchased from Sigma-Aldrich were considered standards. Additionally, the deacetylation degree of chitin (DD) was determined by FTIR in the range between 500-4000 cm^{-1} with a resolution of 4 cm^{-1}.

The reference peak at A1655/A3450 was utilized to compare the absorption peaks of samples [19]. The deacetylation degree (DD) was calculated using the next equation [20]:

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

Where A1655 represents the mean percent absorbance before and after the wavenumber 1655, and A3450 represents the mean percent absorbance at the wavenumber 3450.

Bacterial Strains

Gram-negative bacteria such as *E. faecalis* (ATCC 29212) and *S. epidermidis* (ATCC 12228) and Gram-positive bacteria such as *K. pneumoniae* (ATCC1705) and *P. mirabilis* (ATCC 43071) were obtained from Iranian Industrial Research Organization. Each bacterium was inoculated in an Erlenmeyer flask containing nutrient broth (beef extract 0.5%, NaCl 0.5%, peptone 1%, pH 6, 100 mL), the inoculated flask was then incubated at 37 °C (24 h). To test antibacterial activity, sterile Petri dishes containing Mueller Hinton Agar (MHA, Himedia) medium were incubated at 37 °C (24 h).

Antimicrobial Assays

The disc diffusion method was employed to assess the antimicrobial activity of the samples [21]. Initially, a 0.5 McFarland standard (20 µl) was prepared from fresh bacterial cultures and uniformly spread on plates containing Mueller–Hinton agar. To create chitosan sample discs, the sterile filter paper discs (6 mm) were impregnated with 50 µl of chitosan solution (10 mg/ml). Subsequently, the prepared discs were placed on the plates containing each bacterial strain and incubated at 37°C (24 h). The diameter of the inhibition zones formed around each disc was measured using a metric ruler, serving as an indicator of antimicrobial activity. These measurements were examined as a sign of antimicrobial action. All examinations related to every test organism were repeated three times for each test. Sterile filter paper discs soaked with commercial chitosan solution and acetic acid served as positive controls, while filter paper discs immersed in distilled water were considered as negative controls.

Statistical Analysis

Data were expressed as mean ± standard deviation of the diameter of the inhibition zones. Variance analysis was utilized to determine statistical differences in the diameter of growth inhibition zones among the concentration of extracted chitosan, insect-derived chitosan, and bacterial type. To assess differences among averages at the 0.05 level, the LSD test was applied. All experiments were done in triplicate. Data analysis was performed using SPSS (version 25, Ins. USA).

RESULTS AND DISCUSSION

The quantity of chitin and chitosan extracted from 3 g of dry powder of insects depends on the insect species. Comparatively, the output quantity of chitosan was almost half of that of chitin (Table 1). The amount of chitosan extracted from 3 g of the dry body of German and American cockroaches was 2.95% and 5.8%, respectively. The deacetylation degree (DD) of chitin derived from the samples was computed using FTIR analysis, as shown in Table 2. DD of chitin obtained from German and American cockroaches was 31.5% and 36.8%, respectively. Indeed, the results show that chitosan obtained from German cockroaches is more deacetylated than that from American cockroaches (Table 2).

Table 1. *The yield of chitin and chitosan obtained from 3 grams of powder after the extraction process.*

Insect species	Dry weight (g)	After demineralization (g)	Pure chitin after deproteinization (g)	Pure chitosan after deacetylation (g)
Americana cockroach	3	1.85±0.11 (61.7%)	0.36±0.03 (12%)	0.174±0.01 (5.8%)
German cockroach	3	1.67±0.12 (55.6%)	0.18±0.01 (5.9%)	0.09±0.009 (2.95%)

Table 2. *The deacetylation degree (DD) of the insect's chitin using FTIR analysis at 4,000–500 cm^{-1}.*

Insect species	German cockroach	American cockroach
A1655	0.153	0.126
A3450	0.182	0.149
DD	36.8	31.5

FTIR Analysis

FTIR spectra of cockroaches-derived chitosan were compared with commercial chitosan, as shown in Fig. 1. Three groups indicated the same stretching and bending vibration bands with different infrared spectrum diagrams. Fig.1 shows that peaks are reduced owing to the absorbance and the loss of the acetyl group and deacetylation of chitin. The absorption peaks at 1370–1400 cm^{-1} and 1560–1630 cm^{-1} are attributed to NH2 binding and amid I stretching in C=O, respectively. In the chitosan spectrum (Fig. 1), the peak at 1010–1030 cm^{-1} is ascribed to C–O–C stretching vibrations. Absorption peak related to the C-H stretching, bending vibrations along with the C–O–C stretching vibrations were illustrated in Fig. 1. The absorption bands related to the stretching and C-H bending vibrations are seen in the chitosan spectrum. Peaks of 3427.5, 3452.7, and 3452.4 are related to the hydroxyl group (O-H) in commercial chitosan, American cockroach chitosan, and German cockroach chitosan, respectively.

Sharp peaks at 2931.1 cm^{-1}, 2933.03 cm^{-1}, and 2933.8 cm^{-1} are related to alkanes in spectra of commercial chitosan, American cockroach chitosan, and German cockroach chitosan, respectively. This confirms the correct chemical structure of chitosan, which mainly consists of a C-C single bond. Meanwhile, the sharp peaks at 1649.31 cm^{-1}, 1652.3 cm^{-1}, and 1654.2 cm^{-1} are associated with the N-H bend in spectra of commercial chitosan, American cockroach chitosan, and German cockroach chitosan, respectively.

This medium signal indicates the presence of primary amines. Finally, sharp peaks at 1080.8 cm^{-1}, 1089.8 cm^{-1}, and 1089.7 cm^{-1} are associated with the presence of ether part in spectra of commercial chitosan, American cockroach chitosan, and German cockroach chitosan, respectively. A summary of the FTIR analysis data of commercial chitosan and cockroach-derived chitosan (American and German cockroaches) is shown in Table 3.

Table 3. *Peaks, intensity, functional groups, and shape are present in chitosan.*

FTIR peaks (Commercial)	FTIR peaks (German cockroach)	FTIR peaks (American cockroach)	Functional group	Intensity and shape
3427.536	3452.409	3452.705	-OH (Hydroxyl)	Broad, wide, strong
2931.116	2932.800	2933.034	C-C (Alkane)	Sharp, strong
1649.366	1654.274	1652.303	-NH2 (Amine)	Sharp, medium
1408.336	1402.435	1405.034	C-O-C (Ether)	Sharp, weak
652.009	649.336	654.274	C-H	wide, weak

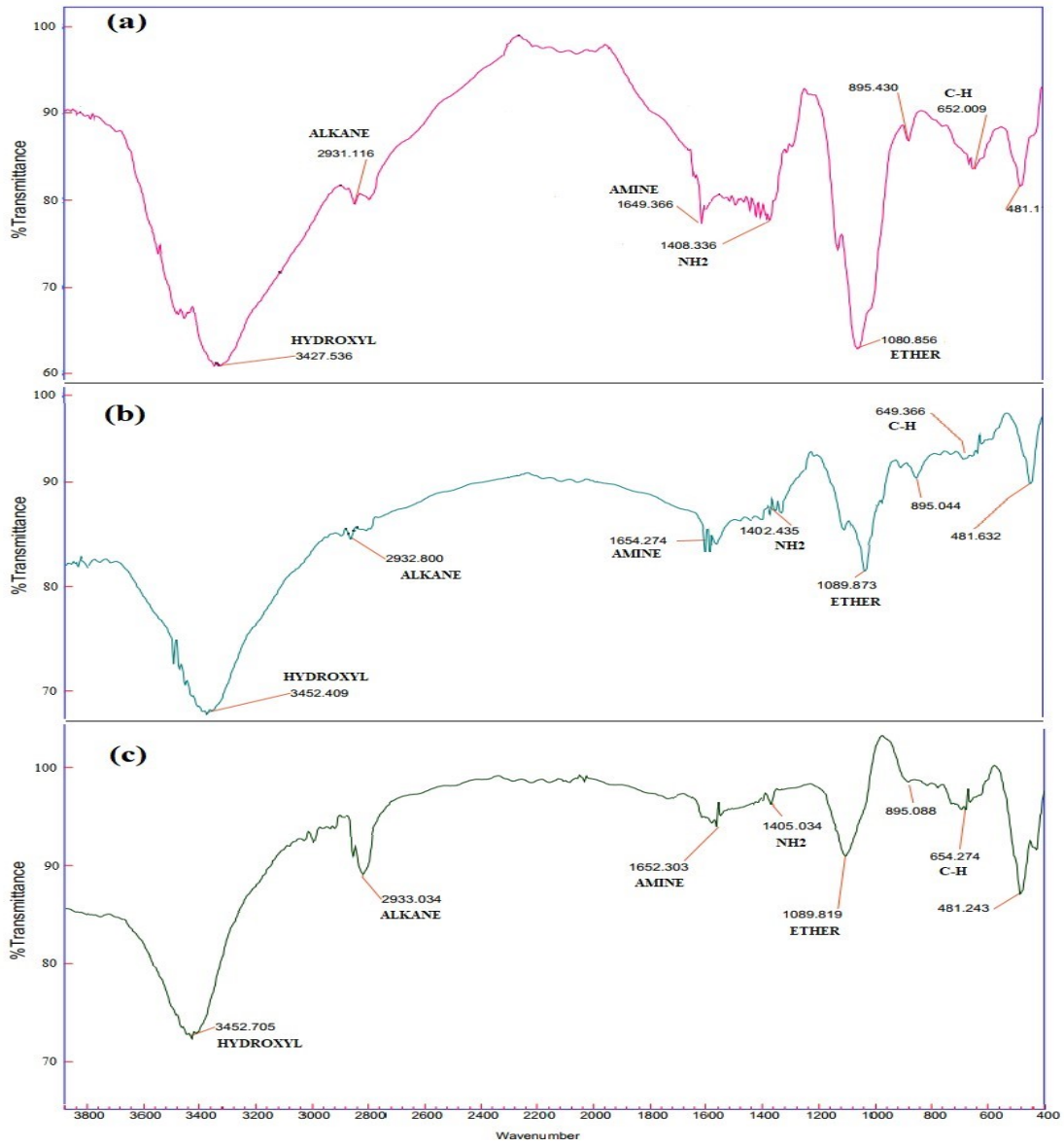

Fig. 1. *FTIR spectra of a) Criterion chitosan, b) German cockroach, c) American cockroach.*

Analysis of Antimicrobial Activities

The inhibition zones related to various concentrations of the insect-derived chitosan are indicated in Table 4. The antibacterial actions of the insect-derived chitosan on Gram-positive bacteria (*S. epidermidis* and *E. faecalis*) and Gram- negative bacteria (*K. pneumoniae* and *P. mirabilis*) are shown in the findings. Investigations showed that the level of antibacterial action of insect-derived chitosan in gram-positive bacteria is different from gram-negative bacteria. In comparison with standard chitosan (p=0.000), the antibacterial action of American cockroach chitosan had the most meaningful effect on a Gram-negative bacterium (*P. mirabilis*) at a 1% concentration. The 1% concentration prepared from American cockroach chitosan (p=0.000) had the most meaningful effect on *P. mirabilis in* comparison to other concentrations prepared from this chitosan. The

antibacterial activity of American cockroach chitosan had the most considerable effect on a Gram-positive bacterium (*S. epidermidis*) at a concentration of 0.1% compared to criterion chitosan (p=0.003). In addition, the results indicated that the concentrations of 1 and 0.01% prepared from American cockroach chitosan with almost similar inhibition zones, had the most meaningful effect on a Gram-negative bacterium (*K. pneumoniae*) in comparison to the concentration of 0.1% (p=0.000). Generally, in comparison with other concentrations prepared from American cockroach chitosan, the 1% concentration had the most meaningful effect on a Gram-negative bacterium (*P. mirabilis*) than other bacteria (p=0.000), as shown in Table 4. In comparison with criterion chitosan (p=0.000), the antibacterial action of German cockroach chitosan had the most meaningful effect on a Gram-negative bacterium (*P. mirabilis*) at the concentrations of 1 and 0.1%. Also, the antibacterial action of German cockroach chitosan had the most meaningful impact on a Gram-negative bacterium (K. pneumoniae) at a 0.01% concentration in comparison to criterion chitosan (p=0.000). In addition, the 0.01% concentration prepared from German cockroach chitosan had the most meaningful impact on a Gram-positive bacterium (*S. epidermidis*) compared to other concentrations (p=0.002) prepared from this chitosan. Generally, in comparison with other concentrations prepared from German cockroach chitosan, the 0.01% concentration had the most meaningful effect on a Gram-negative bacterium (*K. pneumoniae*) compared to other bacteria (p=0.000), as shown in Table 4. As a result, the concentration of 1% prepared from American cockroach chitosan had the most meaningful impact on a Gram-negative bacterium (*P. mirabilis*) in comparison with other bacteria and other concentrations prepared from this chitosan. Also, it is demonstrated that both groups of bacteria are significantly affected by the antibacterial action of German cockroach chitosan at the 0.01% concentration. Indeed, the results illustrated that the insect-derived chitosan significantly inhibited gram-negative bacteria compared with gram-positive bacteria.

Chitin and chitosan from American and German cockroaches were extracted and partially determined in this research. Afterward, the DD was determined, and the bactericidal activity of extracted chitosan was studied. In line with the results of our study, the antimicrobial action of chitosan extracted from these insects was different. Additionally, chitosan extracted from American ad Germany cockroaches exhibited differences in terms of polymerization and crystallinity degrees. Several previous studies have shown that the growth stage affects the yield of chitin obtained from insects, with reported chitin yields ranging from 5.3% to 36.6%. For instance, seven Orthoptera species consisted of 5.3–8.9% [23], *Holotrichia parallela* consisted of 15% [24], *Ranatra linearis* consisted of 15–16% [25], and cicada consisted of 36.6% [26]. The chitin yield extracted from German and American cockroaches was 55.6% and 61.7%, respectively, in our study. Chitosan obtained from shrimp shells was compared with that of cicada slough, silkworm chrysalis, or grasshopper in terms of different features such as surface morphology, rheological and physicochemical characteristics. Based on the reported results, the activity of insect-derived chitosan is thoroughly different from shrimp-obtained chitosan [7]. Additionally, the antimicrobial action of chitosan depends on DD [27]. In comparison to the chitosan of shrimp shells, which has a high DD, insect chitosan is more viscous. Generally, the viscosity of chitosan partly affects its bacteriostatic and bactericidal activity. Indeed, low viscosity is more effective than high viscosity [7, 28]. The antibacterial properties of insect-obtained chitosan are influenced by its different DD. The insect integument, known as an effective alternative source, contains organic materials such as chitin, particularly the cuticle of insects which contains reduced

inorganic content [29]. During the process of deacetylation and demineralization, the yield of chitosan derived from cockroaches was higher compared to that of shrimp [19]. Although cockroaches are an abundant and readily available source of chitin and chitosan, their industrial cultivation is a limiting factor. Similar physiological characteristics have been observed in chitins derived from cockroach species that can be appropriate to produce chitosan. The American cockroach-obtained chitin-chitosan yield was higher than that of the German cockroach, while the DD of chitin/chitosan obtained from the German cockroach was somewhat higher. These findings are attributed to the different molecular weights of chitosan of the two insects, which is in line with previous investigations [15, 30].

Table 4. *Antibacterial activity of insect-derived chitosan on selected bacteria.*

Kind of Selected Bacteria		**Chitosan**	**Diameter of inhibition zone (mm) based on chitosan concentration (%)**		
			1	0.1	0.01
Gram-negative *(G-)*	***Proteus mirabilis***	**American cockroach**	12.2±0.3 [a, b, c]	8.2±0.2	10.2±0.2 [a]
		German cockroach	11.2±0.3	11.2±0.1 [a, b]	8.2±0.1
		Standard (Commercial)	8.2±0.2	9.2±0.3	9.2±0.2
	Klebsiella pneumoniae	**American cockroach**	10.2±0.2 [a]	9.2±0.1	10.2±0.2 [a]
		German cockroach	9.2±0.2	10.2±0.2	11.2±0.3 [a, b, c]
		Standard (Commercial)	8.2±0.1	10.2±0.2	9±0.0
Gram-positive *(G+)*	***Enterococcus faecalis***	**American cockroach**	6.2±0.1	8.2±0.2	8.2±0.1 [b]
		German cockroach	8±0.3	7.2±0.1	8.2±0.2 [b]
		Standard (Commercial)	8.2±0.2	8.2±0.1	8.2±0.2
	Staphylococcus epidermidis	**American cockroach**	8.2±0.2 [a, b]	7.2±0.2 [a]	8.2±0.2
		German cockroach	8.2±0.2 [a]	7.2±0.2 [a]	9.2±0.2 [a, b, c]
		Standard (Commercial)	6.2±0.2	6.2±0.2	8±0.3

*The diameter of the inhibition zone (mm) was computed (mean± SEM) and expressed based on the recommendation of the World Health Organization 2003 [22]. Additionally, all meaningful values of the antimicrobial action of cockroach-derived chitosan in comparison to standard chitosan are displayed in brackets. The best antimicrobial effect for each dose in the zone of inhibition among different chitosan for each bacterium is shown with superscript **a**. The best inhibition zone among the different concentrations of the derived chitosan from the same insect affecting the same bacterium is shown with superscript **b**. The best inhibition zone among the different concentrations and chitosan derived from the different insects affecting each bacterium is shown with superscript **c**. The experiment was performed three times.*

Based on our FTIR results, there are similarities between commercial chitosan and cockroach-derived chitosan in terms of chemical composition and type of bonding. Absorbance peaks at around 1370-1400 and 1560-1630 cm^{-1}, which relate to NH2 in the NHCOCH3 group (amide II band) and C= O in the NHCOCH3 group (amide I band), respectively, were seen in cockroach-derived chitosan. The results are in line with previous studies [15, 24]. The adsorption peaks at around 1010-1030 cm^{-1} were ascribed to C-O-C stretching vibrations in the glucose ring and β (1-4) glycosidic bond in the polysaccharide unit. There is a similarity between the present outcomes and previous reports [15, 24, 30]. Additional broad absorption bands at 2900-3250 cm^{-1} are attributed to symmetric O-H stretching vibrations and alkane caused by strong intermolecular hydrogen bonding of chitosan. The results are in line with the results of other studies [15, 24]. Our results indicated that cockroach-derived chitosan inhibited the growth of both groups of bacteria (Gram-positive and Gram-negative bacteria). In general, molecular weight, degree of deacetylation, pH of the culture medium, or the concentration of chitosan solution are factors that can influence the antibacterial effect of chitosan [6, 31]. Nevertheless, the antimicrobial properties of chitosan depend on numerous factors and may create different results as mentioned by other authors. There is still no consensus on the antibacterial effect of chitosan. Some studies have reported that chitosan has a higher bactericidal action on Gram-positive bacteria compared to Gram-negative bacteria [14, 32]. In contrast, some authors have reported that Gram-negative bacteria are more vulnerable than Gram-positive bacteria due to the hydrophilicity of chitosan [31, 33]. Our results demonstrated that Gram-negative bacteria were more vulnerable to cockroach-derived chitosan, which could be assigned to its high DD. Chitosan with a higher DD carries a more positive charge, allowing it to interact with the bacterial membrane and destroy it. However, some researchers have pointed out that the effect of chitosan on Gram-positive bacteria is greater than Gram-negative bacteria [34, 35]. The antibacterial action of chitosan on both groups of bacteria is somewhat arguable. Contrarily, hydrophilic molecules can penetrate through the outer membrane of gram-negative bacteria, which makes them more vulnerable to chitosan compared to gram-positive bacteria [36]. These findings, obtained through numerous in vitro tests, suggest that Gram-negative bacteria are more susceptible to chitosan than Gram-positive bacteria, leading to more morphological changes upon treatment [37-39]. The consequence is compatible with our results. The charge density on the cell surface is an important factor in the absorption rate of chitosan [40]. Another proposed mechanism by which chitosan exerts its antibacterial effect is through binding to microbial DNA, suppressing mRNA and protein synthesis after entering the nucleus of the microorganisms [38]. Inhibition of spore elements, metal chelation, and binding to nutrients required for microbial growth are other mechanisms [14]. The cell wall of gram-negative bacteria is very complex but thinner than that of gram-positive bacteria, residing in the peptidoglycan layer and suppress the penetration of antibiotics into the cell [16]. This wall consists of a semi-permeable outer membrane, an asymmetric lipid bilayer containing lipopolysaccharide (LPS). The membrane permeability of Gram-negative bacteria is altered due to the electrostatic interaction of negatively charged LPS with chitosan [6, 16].

CONCLUSION

Based on the results, we found that the yield of chitosan and DD relied on the insect species. The species of cockroach from which chitosan is extracted affects the

antibacterial action of chitosan. Gram-negative bacteria were more affected by cockroaches-derived chitosan, especially the American cockroach. This difference is likely attributed to the variations in chitin structure between the two insect species.

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Conflict of Interest. The authors declared that there is no conflict of interest.

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REFERENCES

[1] Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Aguilar GR, Gray A, Han C, Bisignano C, Rao P, Wool E. (2022): Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. The Lancet 399(10325):629-655.

[2] Manniello M, Moretta A, Salvia R, Scieuzo C, Lucchetti D, Vogel H, Sgambato A, Falabella P. (2021): Insect antimicrobial peptides: Potential weapons to counteract the antibiotic resistance. Cell Mol Life Sci 78(9):4259-4282.

[3] Dash M, Chiellini F, Ottenbrite RM, Chiellini E. (2011): Chitosan—A versatile semi-synthetic polymer in biomedical applications. Prog Polym Sci 36(8):981-1014.

[4] Zainol Abidin NA, Kormin F, Zainol Abidin NA, Mohamed Anuar NAF, Abu Bakar MF. (2020): The potential of insects as alternative sources of chitin: An overview on the chemical method of extraction from various sources. Int J Mol Sci 21(14):4978.

[5] Aranaz I, Mengíbar M, Harris R, Paños I, Miralles B, Acosta N, Galed G, Heras Á. (2009): Functional characterization of chitin and chitosan. Curr Chem Biol 3(2):203-230.

[6] Ke C-L, Deng F-S, Chuang C-Y, Lin C-H. (2021): Antimicrobial actions and applications of chitosan. Polymers 13(6):904.

[7] Luo Q, Wang Y, Han Q, Ji L, Zhang H, Fei Z, Wang Y. (2019): Comparison of the physicochemical, rheological, and morphologic properties of chitosan from four insects. Carbohydr Polym 209:266-275.

[8] Zhang M, Haga A, Sekiguchi H, Hirano S. (2000): Structure of insect chitin isolated from beetle larva cuticle and silkworm (Bombyx mori) pupa exuvia. Int J Biol Macromol 27(1):99-105.

[9] Yadollahi M, Farhoudian S, Namazi H. (2015): One-pot synthesis of antibacterial chitosan/silver bio-nanocomposite hydrogel beads as drug delivery systems. Int J Biol Macromol 79:37-43.

[10] Yilmaz Atay H. (2019): Antibacterial activity of chitosan-based systems. Functional chitosan: drug delivery and biomedical applications. p. 457-489.

[11] Liu W, Qin Y, Liu S, Xing R, Yu H, Chen X, Li K, Li P. (2018): Synthesis of C-coordinated O-carboxymethyl chitosan metal complexes and evaluation of their antifungal activity. Sci Rep 8(1):1-10.

[12] Yarahmadi M, Fakhar M, Ebrahimzadeh MA, Chabra A, Rahimi-Esboei B. (2016): The anti-giardial effectiveness of fungal and commercial chitosan against Giardia intestinalis cysts in vitro. J Parasit Dis 40(1):75-80.

[13] Grisin T, Bories C, Bombardi M, Loiseau PM, Rouffiac V, Solgadi A, Mallet J-M, Ponchel G, Bouchemal K. (2017): Supramolecular chitosan micro-platelets synergistically enhance anti-Candida albicans activity of amphotericin B using an immunocompetent murine model. Pharm Res 34(5):1067-1082.
[14] Goy RC, Britto Dd, Assis OB. (2009): A review of the antimicrobial activity of chitosan. Polímeros 19(3):241-247.
[15] Basseri H, Bakhtiyari R, Hashemi SJ, Baniardelani M, Shahraki H, Hosainpour L. (2019): Antibacterial/antifungal activity of extracted chitosan from American cockroach (Dictyoptera: Blattidae) and German cockroach (Blattodea: Blattellidae). J Med Entomol 56(5):1208-1214.
[16] Chang AKT, Frias Jr RR, Alvarez LV, Bigol UG, Guzman JPMD. (2019): Comparative antibacterial activity of commercial chitosan and chitosan extracted from Auricularia sp. Biocatal Agric Biotechnol 17:189-195.
[17] Khan HA, Ahmad A, Mehboob R. (2015): Nosocomial infections and their control strategies. Asian Pac J Trop Biomed 5(7):509-514.
[18] Chang KLB, Tsai G, Lee J, Fu W-R. (1997): Heterogeneous N-deacetylation of chitin in alkaline solution. Carbohydr Res 303(3):327-332.
[19] De Queiroz Antonino RSCM, Lia Fook BRP, de Oliveira Lima VA, de Farias Rached RÍ, Lima EPN, da Silva Lima RJ, Peniche Covas CA, Lia Fook MV. (2017): Preparation and characterization of chitosan obtained from shells of shrimp (Litopenaeus vannamei Boone). Mar Drugs 15(5):141-153.
[20] Zhong Z, Xing R, Liu S, Wang L, Cai S, Li P. (2008): Synthesis of acyl thiourea derivatives of chitosan and their antimicrobial activities in vitro. Carbohydr Res 343(3):566-570.
[21] Hudzicki J. (2009): Kirby-Bauer disk diffusion susceptibility test protocol. American society for microbiology 15:55-63.
[22] WorldHealthOrganization. (2003): Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world. Geneva: World Health Organization; p. No. WHO/CDS/CSR/RMD/2003.6359.
[23] Kaya M, Erdogan S, Mol A, Baran T. (2015): Comparison of chitin structures isolated from seven Orthoptera species. Int J Biol Macromol 72:797-805.
[24] Liu S, Sun J, Yu L, Zhang C, Bi J, Zhu F, Qu M, Jiang C, Yang Q. (2012): Extraction and characterization of chitin from the beetle Holotrichia parallela Motschulsky. Molecules 17(4):4604-4611.
[25] Kaya M, Baran T, Mentes A, Asaroglu M, Sezen G, Tozak KO. (2014): Extraction and characterization of α-chitin and chitosan from six different aquatic invertebrates. Food Biophys 9:145-157.
[26] Sajomsang W, Gonil P. (2010): Preparation and characterization of α-chitin from cicada sloughs. Mater Sci Eng C 30(3):357-363.
[27] Jung EJ, Youn DK, Lee SH, No HK, Ha JG, Prinyawiwatkul W. (2010): Antibacterial activity of chitosans with different degrees of deacetylation and viscosities. Int J Food Sci Technol 45(4):676-682.
[28] Dragland IS, Rukke HV, Stenhagen IS, Lönn-Stensrud J, Kopperud HM. (2016): Antibacterial and antibiofilm effect of low viscosity chitosan against Staphylococcus epidermidis. Int J Microbiol 2016:1-7.
[29] Kaya M, Baran T, Erdoğan S, Menteş A, Özüsağlam MA, Çakmak YS. (2014): Physicochemical comparison of chitin and chitosan obtained from larvae and adult Colorado potato beetle (Leptinotarsa decemlineata). Mater Sci Eng C 45:72-81.
[30] Badawy RM, Mohamed HI. (2015): Chitin extration, composition of different six insect species and their comparable characteristics with that of the shrimp. J Am Sci 11(6):127-134.
[31] Vinsova J, Vavrikova E. (2011): Chitosan derivatives with antimicrobial, antitumour and antioxidant activities-a review. Curr Pharm Des 17(32):3596-3607.

[32] Zhao G, Hu C, Xue Y. (2018): In vitro evaluation of chitosan-coated liposome containing both coenzyme Q10 and alpha-lipoic acid: Cytotoxicity, antioxidant activity, and antimicrobial activity. J Cosmet Dermatol 17(2):258-262.
[33] Raafat D, Sahl HG. (2009): Chitosan and its antimicrobial potential–a critical literature survey. Microb Biotechnol 2(2):186-201.
[34] Coma V, Deschamps A, Martial-Gros A. (2003): Bioactive packaging materials from edible chitosan polymer—antimicrobial activity assessment on dairy-related contaminants. J Food Sci 68(9):2788-2792.
[35] Dutta P, Tripathi S, Mehrotra G, Dutta J. (2009): Perspectives for chitosan based antimicrobial films in food applications. Food Chem 114(4):1173-1182.
[36] Chung Y-C, Su YP, Chen C-C, Jia G, Wang HL, Wu JG, Lin JG. (2004): Relationship between antibacterial activity of chitosan and surface characteristics of cell wall. Acta Pharmacol Sin 25(7):932-936.
[37] Eldin MM, Soliman E, Hashem A, Tamer T. (2008): Antibacterial activity of chitosan chemically modified with new technique. Trends Biomater Artif Organs 22(3):125-137.
[38] Šimůnek J, Tishchenko G, Hodrová B, Bartoňová H. (2006): Effect of chitosan on the growth of human colonic bacteria. Folia Microbiol 51(4):306-308.
[39] Eaton P, Fernandes JC, Pereira E, Pintado ME, Malcata FX. (2008): Atomic force microscopy study of the antibacterial effects of chitosans on Escherichia coli and Staphylococcus aureus. Ultramicroscopy 108(10):1128-1134.
[40] Másson M, Holappa J, Hjálmarsdóttir M, Rúnarsson ÖV, Nevalainen T, Järvinen T. (2008): Antimicrobial activity of piperazine derivatives of chitosan. Carbohydr Polym 74(3):566-571.