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Bioactivity of Chitosan Extracted from the Red Palm Weevil, *Rhynchophorus Ferrugineus* Olivier (Coleoptera: Dryophthoridae)

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Abstract

he present study aimed at investigating the antibacterial and antioxidant activities of chitosan extracted from Rhynchophorus ferrugineus adults. The Fourier transform infrared (FT-IR) analysis exhibited an absorption peak of amide I band at 1635 cm−1, respectively. The degree of deacetylations (DDA) recorded 74.25%, respectively. The X-ray diffraction (XRD) analysis showed two sharp peaks at 14.76 and 19.98º and two faints at 10.02º and 26.48º diffraction peaks. Also, scanning electron microscope (SEM) studies for the extracted chitosan surface morphology exhibited a regular surface, soft structure with nanopores and consisted of long and tidy nanofibers that adhered to each other and randomly scattered big nanopores. On the other hand, results of antibacterial activity indicated that tested Gram-positive bacterial strains were more sensitive to R. ferrugineus extracted chitosan than those of Gram-negative ones. The highest antibacterial activity (24.6±0.5 and 21.3±0.5 mm inhibition zones) recorded against Escherichia coli and Enterobacter hormaechei at 750 µg/ml, respectively. In addition, 2,2-Diphenyl-1-picryl Hydrazine, or hydrazyl-hydrate free radical (DPPH) screening test showed the good antioxidant effects of the R. ferrugineus extracted chitosan) and their scavenging activity depending on the concentration used. At 1000 and 1.95 µg/ml, R. ferrugineus extracted chitosan was found to be active radical scavenger (61.14 and 21.92%), respectively. Based on these results, chitosan extracted from R. ferrugineus adults can play an important role as potential antibacterial and antioxidant agent.

Keywords: Chitosan; *Rhynchophorus ferrugineus;* antibacterial activity; antioxidant

1. Introduction

Natural products are a large group of various chemical entities, which have a broad bioactivity that found manifold uses notably in modern human medicine (Amer *et al,* 2019a). Chitosan has a broad antibacterial activity and considered to be one of the most important properties, conformable directly to its possible bio implementations (Zhao and Xia, 2006; Anna *et al,* 2020). In 1979, Allan and Hadwiger, reported the broad-spectrum antimicrobial effects of chitosan for the first time and hence many studies have been performed. Activity of chitosan against several microbes depends on the target organism and intrinsic factors of chitosan as molecular weight, positive charge density, physical state factors, as well as environmental factors as ionic forces, pH, temperature, and time (Kong *et al,* 2010; Badawy and Rabea, 2011). In the last few decades, chitosan usually extracted from crustaceans' shell through four steps (El-Mehdawy *et al,* 2022). Class Insecta are the largest group of organisms, representing about 80.0% of the all-known fauna and considered as unexplored and unexploited source of many bioactive compounds for modernistic medicine (Bulet *et al,* 2004; Amer *et al,* 2019b). Chitosan can be extracted from insects through deproteinization and demineralization only (Gobinath *et al,* 2021; Ibram *et al,* 2021).

Since few data concerning with antibacterial and antioxidant activities of insect-derived chitosan are obtainable, the present study was performed to investigate chitosan extracted from the red palm weevil, *Rhynchophorus ferrugineus* adult (Dryophthoridae) as a potential antibacterial agent giving preservation and barring from diseases.

2. Experimental

1. Tested Rhynchophorus ferrugineus:

Larvae, cocoon and adult stages of red palm weevil, *R. ferrugineus* were collected from Central

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Laboratory for Date Palm Research and Development, Agricultural Research Center, Giza, Egypt and was reared for five generations in insectary, Animal house, Zoology Department, Faculty of Science, Al-Azhar University (Cairo), under controlled conditions of temperature (25±2°C), relative humidity (60-70%) and photoperiods (12-12 hours light-dark rhythm). The standard rearing procedure described by Shahina *et al,* (2009) was applied to provide individuals which needed for the bioassay. Briefly, three different media were used for feeding and egg production; cotton wool pieces saturated with 20% honey; five pieces of apple and five pieces of sugarcane (5×2 cm). About 50 pairs of *R. ferrugineus* were kept together in a 300 ml glass jars and were tightly covered with perforated caps having the above-mentioned medium separately. Eggs laid were recorded daily and medium was changed after 2-3 days. After emergence from eggs (2-5 days) soft whitish larvae were transferred in cotton wools saturated with 60.0% honey, fresh apple, fresh sugarcane stem (20 cm) with the help of fine hairbrush. When last larval stage reached at the bottom of stem and about to pupate (pre-pupal stage) it was transferred to fresh sugarcane stem. After 3 days of pre-pupal stage, they constructed a pupal case (cocoons) which was dark brown in color. Adult came out by rupturing the cocoons.

2. Process of chitin isolation and chitosan extraction:

Extraction of chitosan was performed according to the method of Kaya *et al,* (2015) where, cleaned adults of *R. ferrugineus* left to dry at room temperature and pulverized by crushing in a mortar. A sample of 40 g used for chitin isolation process. Demineralization carried out using 400 ml 1M HCL for 12 hours at room temperature. Deproteinization performed by using 5% NaOH at 90°C for 8 hrs. for decoloration, sample passed through a mixture of chloroform, methanol and distilled water in the ratio of (1:2:4) at room temperature for 6 hrs. and rinsed with distilled water. Deacetylation carried out using 50.0% NaOH at 90°C for 8 hrs.

2.1. Characterization of *R. ferrugineus* extracted chitosan

2.1.1. Fourier transform infrared spectroscopy (FT-IR)

The FT-IR analysis was carried out using JASCO FTIR-6200 instrument at Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt. The degree of deacetylation (DDA) of chitosan was calculated (Akila 2014) as:

DDA% = 100- [(A1655/ A3450) x 100/1.33]

Where:

A_{1655}: Absorption of band at 1655 cm^{-1},

A_{3450}: Absorption of band at 3450 cm^{-1},

A_{Band}= - log (Transmittance)…

2.2.2. X-Ray diffraction (XRD)

Phase identification, purity, relative crystallinity and crystallite size of *R. ferrugineus* extracted chitosan were analyzed by X-ray diffractometer (Panalytical X'Pert Pro, Netherland) using Cu-Kα as a source of radiation and nickel filter at a scanning speed of 2° min^{-1} in the 2θ range from 4-70° at Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt. The crystalline index (CrI) values were calculated from XRD data according to (Marei *et al*, 2016):

$CrI_{110} = [(I_{110} - I_{am}) / I_{110}] \times 100$

Where:

I_{110}: maximum intensity at $2\theta \cong 20°$,

I_{am}: intensity of amorphous diffraction at $2\theta \cong 16°$.

2.2.3. Scanning Electron Microscopy (SEM):

The surface morphology and microstructure of chitosan was examined using a scanning electron microscopy (JEOL-JSM-5500 LV) performed to study surface morphology and microstructure of *R. ferrugineus* extracted chitosan at the Regional Center of Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

3. Bioactivity of prepared chitosan

3.1. Antibacterial bioassay

Six pathogenic bacteria species were used for the antibacterial assay; Enterococcus faecalis, Staphylococcus aureus, and Staphylococcus haemolyticus as Gram-positive bacterial species, while Escherichia coli, Enterobacter hormaechei and Klebsiella pneumoniae as Gram-negative bacterial species. The antibacterial assay was carried out using Agar well diffusion method at Microbiology Department, Faculty of Science, Al-Azhar University using a standard procedure described by (Oluwafemi and Debiri, 2008). Concentrations of 250, 375, 500, and 750 µg/ml from R. ferrugineus extracted chitosan were poured in the prepared holes using an automatic micropipette. Penicillin was used as a standard wide spectrum antibacterial agent to compare test samples.

Minimal inhibitory concentrations (MICs) of AgNPs against *E. faecalis, S. aureus, S. haemolyticus, E. coli, E. hormaechei and K. pneumoniae*were assessed using the microbroth dilution method (Travnickova *et al,* 2019). Micro-dilution of overnight grown culture strains was cultured using Muller-Hinton broth medium in 96-well plates. Different concentrations of *R. ferrugineus* extracted chitosan (250, 375, 500, and 750 µg/mL) were added and plates were incubated at 37 °C overnight. Then,

30µl resazurin solution (0.1 mg/mL) was added to each well and incubated at 37 °C for 2 h. Visual change in culture color from blue dye to pink within viable cells was assessed (Shehabeldine and Hasanin, 2019).

3.2. Antioxidant activity of prepared chitosan by DPPH radical scavenging method

Free radical scavenging activity of *R. ferrugineus* extracted chitosan was measured by 2, 2- diphenyl-1-picryl hydrazine (DPPH) at Microbiology Department, Faculty of Science, Al-Azhar University. In brief, 0.1 mM solution of DPPH in ethanol was prepared and added to 3 ml of chitosan. The mixture was shaken vigorously and allowed to stand at room temp for 30 min. then, absorbance was measured at 517 nm by using spectrophotometer (UV-VIS Milton Roy). Reference standard compound being used was ascorbic acid and experiment was done in triplicate (Shimada *et al*, 1992).

The percent DPPH scavenging effect was calculated as DPPH scavenging effect (%) or percent inhibition = $[(A_0 - A_1) / A_0] \times 100$

Where:

A0: is absorbance of the standard.

A1: is absorbance of the extract.

Results

Deacetylation process of chitin was ensured by appearance of dark purple color indicated the success of chitosan extraction.

1. Characterization of *R. ferrugineus* extracted chitosan

The FT-IR revealed that, chitosan extracted from the *R. ferrugineus* exhibited a wave number at 3417 cm^{-1}, corresponding to the stretching vibration of –OH, the extension vibration of N–H, and intermolecular hydrogen bonds of polysaccharide. The absorption bands at 2924 cm^{-1} were assigned to the asymmetric stretching vibration (CH_2) in CH_2OH group. The absorption peak of amide I band (due to -C=O stretching of hydrogen bonded -C=O-$NHCOCH_3$ group) was observed at around 1635 cm^{-1}. The bands at 1420 and 1381 cm^{-1} were attributed to the bending (CH_2) in CH_2OH group and symmetric bending (CH_3) in $NHCOCH_3$ group, respectively. Wave number at 1265 cm^{-1} was related to the complex vibrations of NHCO group (Amide III band). The C–O–C symmetric stretching vibration in the glycosidic linkage was examined at a wave number of 1157 cm^{-1}. The stretching vibration (C-O) in OH group was observed at 1111 cm^{-1}. The stretching vibration (C–O) in secondary OH group was observed at 1026 cm^{-1}. In addition, wave number at 895 cm^{-1} was ascribed to the C–H out of plane vibration (Pyranose ring skeletal vibrations). Degree of deacetylation (DDA) recorded 74.25 %, respectively (Figure 1).

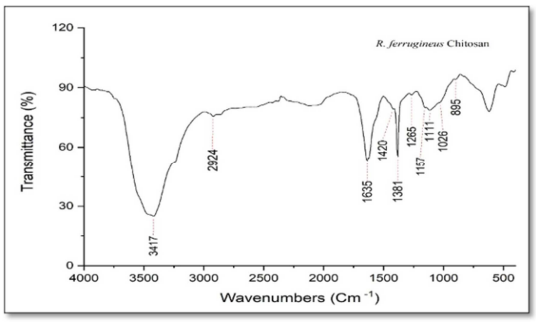

Figure 1: The FT-IR spectra of the chitosan prepared from the *V. Orientalis*

Also, the X-ray diffraction analysis of *R. ferrugineus* extracted chitosan showed two sharp peaks at 14.76 and 19.98º and two faints at 10.02º and 26.48º diffraction peaks. The crystalline index (CrI) recorded 81.8 %, respectively (Figure 2).

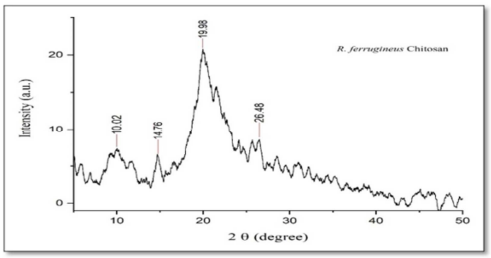

Figure 2: The XRD pattern of *R. ferrugineus* extracted chitosan

On the other hand, SEM studies exhibited a regular surface of *R. ferrugineus* extracted chitosan, soft structure with nanopores and consisted of long and tidy nanofibers that adhered to each other and randomly scattered big nanopores are observed (Figure 3).

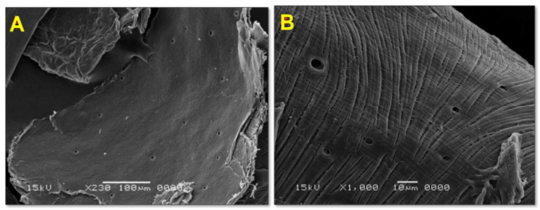

Figure 3: The SEM images showing the surface morphologies of *R. ferrugineus* extracted chitosan

2. Antibacterial activity of tested chitosan

Data in table 1 showed the antibacterial activity of *R. ferrugineus* extracted chitosan against Gram-positive bacterial strains. Data revealed that, growth-inhibition zones in mm recorded by *R. ferrugineus* extracted chitosan were 24.0±1.7 and 19.3±1.15 against *Enterococcus faecalis* at 750 and 500 µg/ml, respectively. Also, at 750 µg/ml *R. ferrugineus* extracted chitosan recorded 24.6±0.5 and 20.0±0.0 mm inhibition zones against *Staphylococcus aureus* and *S. haemolyticus,* respectively.

Table 1: Antibacterial activity (indicated by growth-inhibition zone) of *R. ferrugineus* extracted chitosan against Gram-positive bacterial strains.

Bacterial strains (Gram +ve)	Conc. (µg/ml)	Growth-inhibition zone in mm*	
		Tested chitosan	Standard (Penicillin)
Enterococcus faecalis	250	NA	31.6±1.15
	375	19.0±1.7	
	500	19.3±1.15	
	750	24.0±1.7	
Staphylococcus aureus	250	12.3±0.5	20.3±0.5
	375	16.6±0.5	
	500	21.3±1.15	
	750	24.6±0.5	
Staphylococcus haemolyticus	250	NA	NA
	375	11.3±1.15	
	500	13.6±0.5	
	750	20.0±0.0	

*: inhibition zone in mm ± standard deviation (SD) beyond well diameter (6mm). *NA*: No activity

On the other hand, *R. ferrugineus* extracted chitosan exhibited a variable antibacterial activity against Gram-ve bacterial strains tested depending on concentration used. At 750 µg/ml, tested chitosan recorded growth-inhibition zones equal to 24.6±0.5, 21.3±0.5 and 19.6±0.5 mm against *Escherichia coli, Enterobacter hormaechei* and *Klebsiella pneumoniae,* respectively. Whereas no activity recorded by *R. ferrugineus* extracted chitosan against all Gram -ve bacterial strains tested at 250 µg/ml (Table 2).

Table 2: Antibacterial activity (indicated by growth-inhibition zone) of *R. ferrugineus* extracted chitosan against Gram-negative bacterial strains

Bacterial strains (Gram -ve)	Conc. (µg/ml)	Growth-inhibition zone in mm*	
		Tested chitosan	Standard (Penicillin)
Escherichia coli	250	NA	NA
	375	14.6±0.5	
	500	23.0±1.0	
	750	24.6±0.5	
Enterobacter hormaechei	250	NA	10.0±0.0
	375	NA	
	500	17.3±0.5	
	750	21.3±0.5	
Klebsiella pneumoniae	250	NA	NA
	375	13.3±0.5	
	500	14.3±0.5	
	750	19.6±0.5	

See footnote of table 1

The Minimum Inhibitory Concentration (MIC) of *R. ferrugineus* extracted chitosan was 250 µg/ml against *S. aureus* only, but it was increased in case of *E. faecalis, S. haemolyticus, E. coli* and *K. pneumoniae* to 375 µg/ml also increase to 500 µg/ml against *E. hormaechei* only (Table 3).

Table 3: Minimal Inhibitory Concentrations (MIC) of different concentrations in µg/ml of R. ferrugineus extracted chitosan against different strains of bacteria.

Conc. (µg/ml)	Bacterial strains					
	E. faecalis	*S. aureus*	*S. haemolyticus*	*E. coli*	*E. hormaechei*	*K. pneumoniae*
250	*NA*	12.3±0.5	*NA*	*NA*	*NA*	*NA*
375	19.0±1.7	16.6±0.5	11.3±1.15	14.6±0.5	*NA*	13.3±0.5
500	19.3±1.15	21.3±1.15	13.6±0.5	23.0±1.0	17.3±0.5	14.3±0.5
750	24.0±1.7	24.6±0.5	20.0±0.0	24.6±0.5	21.3±0.5	19.6±0.5

3. Antioxidant activity of chitosan:

Results presented in table 4 showed the antioxidant activity of chitosan extracted from *R. ferrugineus* opposite ascorbic acid used as standard at the same concentrations. DPPH radical screening test showed the good antioxidant effects of the R. ferrugineus extracted chitosan. At 1000 µg/ml, *R. ferrugineus* extracted chitosan was found to be active radical scavenger (61.14 %), respectively. While ascorbic acid recorded 99.92% scavenging effect at the same concentration. At 1.95 µg/ml, *R. ferrugineus* extracted chitosan recorded DPPH% equal to 21.92, compared with 26.02% for ascorbic acid (standard), respectively (Figures 4 and 5).

Table 4: The DPPH radical scavenging activity of the *R. ferrugineus* extracted chitosan against the standard

Conc. (µg/ml)	*R. ferrugineus* extracted chitosan		Ascorbic acid (standard)	
	Optical density	DPPH %	Optical density	DPPH %
1000	0.521	61.14	0.001	99.92
500	0.611	54.43	0.004	99.70
250	0.724	46.01	0.01	99.25
125	0.948	29.30	0.125	90.67
62.5	0.973	27.44	0.264	80.31
31.25	0.99	26.17	0.398	70.32
15.75	1.018	24.08	0.589	56.07

7.8	1.02	23.93	0.698	47.94
3.9	1.03	23.19	0.872	34.97
1.95	1.047	21.92	0.992	26.02

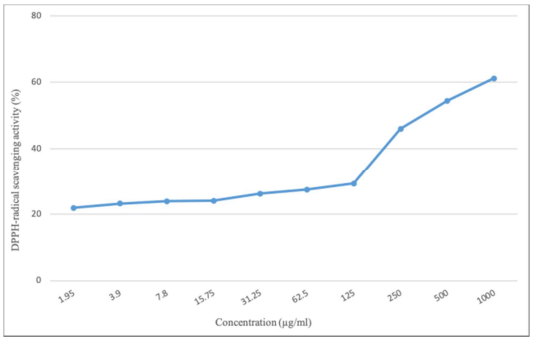

Figure 4: Antioxidant activity of *R. ferrugineus* extracted chitosan

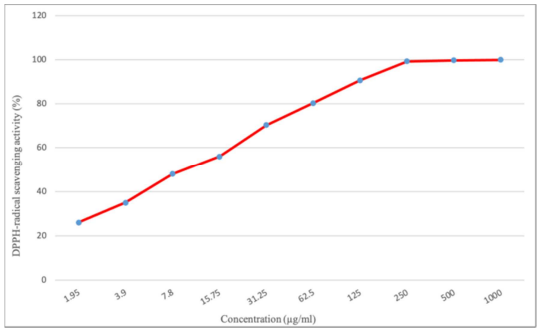

Figure 5: Antioxidant activity of ascorbic acid (standard)

Discussion:

1. Characterization of chitosan:

The FT-IR analysis showed that chitosan extracted from *R. ferrugineus* adults exhibited an absorption peak of amide I band (due to -C=O stretching of hydrogen bonded -C=O-NHCOCH$_3$ group) at 1635 cm^{-1}, respectively. The degree of deacetylations (DDA) recorded 74.25 %, respectively. Similar results were recorded by Rady *et al,* (2018) for chitosan derived from *Vespa orientalis* where, the DDA recorded about 93.0%. Battampara *et al,* (2020) where, DDA% of chitosan extracted from silkworm pupae and eggshell recorded 67.0 and 59.0% and Ibram *et al,* (2021) for chitosan extracted from *Schistocerca gregaria, Apis mellifera* and *Calosoma rugosa* (DDA ranged from 95.0 to 98.0%).

On the other hand, X-ray diffraction analysis of *R. ferrugineus* extracted chitosan showed two sharp peaks at 14.76 and 19.98º and two faints at 10.02º and 26.48º diffraction peaks, similar peaks were recorded by Wang *et al,* 2013, Kaya *et al,* 2014 and Rady *et al,* (2018). The crystalline index (CrI) of *R. ferrugineus* extracted chitosan recorded 81.8 %, like that obtained by Battampara *et al,* (2020) where, crystallinity of silkworm pupae and eggshell chitosan recorded 48.0 and 38.0%, respectively. Also, SEM studies for extracted chitosan surface morphology exhibited a regular surface, soft structure with nanopores and consisted of long and tidy nanofibers that adhered to each other and randomly scattered big nanopores. These results support that reported by Kaya *et al,* (2014) for chitosan extracted from *Leptinotarsa decemlineata* larvae and adults and Marei *et al,* (2016) for chitosan extracted from *Schistocerca gregaria, Apis mellifera* and *Calosoma rugosa.*

2. Antibacterial activity of tested chitosan:

The present work showed that, *R. ferrugineus* extracted chitosan recorded a variable antibacterial activity against both tested Gram-positive and Gram-negative bacterial strains depending on concentration and bacterial strain used. The highest antibacterial activity (24.6±0.5 and 21.3±0.5 mm inhibition zones) recorded against *E. coli* and *E. hormaechei* at 750 µg/ml, respectively. These results are in agreement with the previously recorded by Prabu and Natarajan, (2012) where, antimicrobial activity of chitosan isolated from *Podophthalmus vigil* was strengthened by increasing concentration, furthermore with Kaya *et al,* (2015) where, antimicrobial properties of chitosan obtained from *Calliptamus barbarus* and *Oedaleus decorus* showed a significant antimicrobial activity against *Listeria monocytogenes*, *B. subtilis*, *Sa. enteritidis* and *Yersinia enterocolitica,* Varun *et al,* (2017) where, inhibitions zones (mm) recorded by chitosan and chito-oligomers extracted from the shrimp shell waste against *En. aerogen, En. faecalis, E. coli* and *S. aureus* were 13±0.20, 11.5±0.4, 10.7±0.2 and 10.7±0.3, respectively and Aloucheh *et al,* (2019) where, gram-positive bacterial strains were more sensitive to chitosan isolated from the aquatic beetles (Hydraenidae) in comparison with gram-negative strains.

3. Antioxidant activity of tested chitosan:

Chitosan extracted from *R. ferrugineus* adults exhibited an effective antioxidant activity against DPPH and their scavenging activity depending in concentration used. At 1000 and 1.95 µg/ml, *R. ferrugineus* extracted chitosan was found to be active radical scavenger (61.14 and 21.92%), respectively. These results confirm results of Kaya *et al,* (2014) using chitosan extracted from

Leptinotarsa decemlineata larva and adult, where DPPH radical scavenging activity test showed that inhibitory activity recorded 54.43% of the DPPH radicals by chitosan extracted from adult at 5.0 mg/ml, compared with 33.05% recorded by chitosan extracted from larvae at the same concentration, respectively, Ozusaglam *et al*, (2016) using chitosan extracted from *Ceriodaphnia quadrangular* that inhibited 35.83% of the 1, 1-diphenyl-2-picrylhydrazyl radicals at 5.0 mg/ml and Srinivasan *et al,* (2018) using chitin and chitosan obtained from *Penaeus monodon* shells, where the scavenging activity ranged from 5.41 to 59.02% in chitin and 11.35 to 68.25% in chitosan, respectively.

Conclusion:

Results of antibacterial activity indicated that tested Gram-positive bacterial strains were more sensitive to *R. ferrugineus* extracted chitosan than those of Gram-negative. Also, tested chitosan showed an antioxidant activity but lower than that recorded by ascorbic acid. Thus, chitosan extracted from *R. ferrugineus* adults can play a role as potential antibacterial and antioxidant agent however, more studies concerning with activity of tested chitosan against different microbes are needed.

Conflicts of interest

There are no conflicts to declare.

References

Akila, R M, (2014). Fermentative production of fungal Chitosan, a versatile biopolymer (perspectives and its applications). *Adv. Appl. Sci. Res.*, 5: 4: 157-170.

Allan, CR, Hadwiger, LA, (1979). The fungicidal effect of chitosan on fungi of varying cell wall composition. *Exp. Mycol.*, 3: 285-287. https://doi.org/10.1016/S0147-5975(79)80054-7

Aloucheh, RM, Baris, O, Asadi, A, Golamzadeh, S, Sadeghi, MK, (2019). Characterization of aquatic beetles shells (Hydraenidae family) derived chitosan and its application in order to eliminate the environmental pollutant bacteria. *Anthropog. Pollut. J*., 3,2: 43-48. http://dx.doi.org/10.22034/ap.2019.668483

Amer, MS, Hammad, KM, Shehata, AZI, Hasaballah, AI, Zidan, MM, (2019a). Antimicrobial and antiviral activity of *Lucilia sericata*, *Chrysomya albiceps* (Diptera: Calliphoridae) and *Musca domestica* (Diptera: Muscidae) whole body extract. *Egypt. Acad. J. Biolog. Sci. (A. Entomology)*, 12,2: 19-33. https://dx.doi.org/10.21608/eajbsa.2019.28791

Amer, MS, Hammad, KM, Shehata, AZI, Hasaballah, AI, Zidan, MM, (2019b). Electrophoretic protein and amino acid analysis of *Lucilia sericata*, *Chrysomya albiceps* (Diptera: Calliphoridae) and *Musca domestica* (Diptera: Muscidae) larval excretion/secretion. *Egypt. Acad. J. Biolog. Sci.*, 11,1: 117-130. https://dx.doi.org/10.21608/eajbsc.2019.28687

Anna, B, Solaiman, D, Alexey, S, Sali, D, (2020). Pharmacological and biological effects of chitosan. *Research J. Pharm. and Tech.,* 13,2:1043-1049. https://doi.org/10.5958/0974-360X.2020.00192.4

Badawy, M E I, Rabea, E I, (2011). A biopolymer chitosan and its derivatives as promising antimicrobial agents against plant pathogens and their applications in crop protection. *Int. J. Carbohydr. Chem.*, 460381: 1-29. https://doi.org/10.1155/2011/460381

Battampara, P, Sathish, TN, Reddy, R, Guna, V, Nagananda, GS, Reddy, N, Ramesha, BS, Maharaddi, VH, Rao, AP, Ravikumar, HN, Biradar, A, Radhakrishna, PG, (2020). Properties of chitin and chitosan extracted from silkworm pupae and egg shells. *Int. J. Biol. Macromol.*, 161: 1296-1304. https://doi.org/10.1016/j.ijbiomac.2020.07.161

Bulet, P, Stocklin, R, Menin, L, (2004). Anti-microbial peptides: from invertebrates to vertebrates. *Immunol. Rev.*, 198: 169-184. https://doi.org/10.1111/j.0105-2896.2004.0124.x

El-Mehdawy, AA, Koriem, M, Amin, RM, Shehata, AZI, El-Naggar, HA, (2022). Green synthesis of silver nanoparticles using chitosan extracted from *Penaeus indicus* and its potential activity as aquatic larvicidal agent of *Culex pipens*. *Egypt. J. Aquat. Biol. Fish*, 26,1: 425-442. https://dx.doi.org/10.21608/ejabf.2022.219887

Gobinath, T, Thamizhselvan, S, Ramakrishnan, A, Ravichandran, S, (2021). Preparation and characterization of chitosan from *Perna viridis* (Linnaeus, 1758) shell waste as raw material. *Research J. Pharm. and Tech.,* 14,5:2757-2. https://doi.org/10.52711/0974-360X.2021.00486

Ibram, A, Ionescu, AM, Cadar, E, (2021). Comparison of extraction methods of chitin and chitosan from different sources. *Eur. J. Med. Sci.*, 5,1: 44-56. https://revistia.org/files/articles/ejnm_v2_i2_19/Ibram.pdf

Kaya, M, Baran, T, Erdogan, S, Mentes. A, Ozusağlam, 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. https://doi.org/10.1016/j.msec.2014.09.004

Kaya, M, Baran, T, Ozusaglam, MA, Cakmak, YS, Tozak, KO, Mol, A, Mentes, A, Sezen, G, (2015). Extraction and characterization of chitin and chitosan with antimicrobial and antioxidant activities from cosmopolitan Orthoptera species (Insecta). *Biotechnol. Bioprocess Eng.*, 20: 168-179. https://doi.org/10.1007/s12257-014-0391-z

Kong, M, Chen, X G, Xing, K, Park, H J (2010). Antimicrobial properties of chitosan and mode of action: A state of the art review. *Int. J. Food. Microbiol.*, 144,1: 51-63. https://doi.org/10.1016/j.ijfoodmicro.2010.09.012

Marei, N H, Abd El Samiee, E, Salah, T, Saad, G R, Elwahy, A H M, (2016). Isolation and characterization of chitosan from different local insects in Egypt. *Int. J. Biol. Macromol.*, 82: 871-877. https://doi.org/10.1016/j.ijbiomac.2015.10.024

Oluwafemi, F, Debiri, F, (2008). Antimicrobial effect of *Phyllanthus amarus* and *Parquetina nigrescens* on *Salmonella typhi*. *Afr. J. Biomed. Res.*, 11: 215-219. https://doi.org/10.4314/ajbr.v11i2.50712

Ozusaglam, M A, Cakmak, Y S, Kaya, M, Erdogan, S, Baran, T, Mentes, A, Saman, I, (2016). Antimicrobial and antioxidant properties of *Ceriodaphnia quadrangula* Ephippia chitosan. *Rom. Biotechnol. Lett.*, 21,5: 11881-11890.

Prabu, K, Natarajan, E, (2012). In vitro antimicrobial and antioxidant activity of chitosan isolated from *Podophthalmus vigil*. *J. Appl. Pharm. Sci.*, 2,9: 75-82. http://dx.doi.org/10.7324/JAPS.2012.2916

Rady, M H, Essa, E E, Mamoun, S A M, Momen, S A A, Salama, M S, Barakat, E M S, Abou-Elmagd, W S I, (2018). Characterization and solubilization of chitosan from the oriental hornet (*Vespa orientalis*). *J. Egypt. Soc. Parasitol.*, 48,3: 669-676. https://dx.doi.org/10.21608/jesp.2018.76586

Shahina, F, Salma, J, Mehreen, G, Bhatti, M I, Tabassum, K. A, (2009). Rearing of *Rhynchophorus ferrugineus* in laboratory and field conditions for carrying out various efficacy studies using EPNs. *Pak. J. Nematol.*, 27, 2: 219-228.

Shimada, K, Fujikawa, K, Yahara, K, Nakamura, T, (1992). Antioxidative properties of xanthone on the autoxidation of soybean in cyclodextrin emulsion. *J. Agric. Food Chem.*, 40,6: 945-948. https://doi.org/10.1021/jf00018a005

Srinivasan, H, Kanayairam, V, Ravichandran, R, (2018). Chitin and chitosan preparation from shrimp shells *Penaeus monodon* and its human ovarian cancer cell line, PA-1. *Int. J. Biol. Macromol.*, 107: 662-667. https://doi.org/10.1016/j.ijbiomac.2017.09.035

Varun, T K, Senani, S, Jayapal, N, Chikkerur, J, Roy, S, Tekulapally, V B, Gautam, M, Kumar, N, (2017). Extraction of chitosan and its oligomers from shrimp shell waste, their characterization and antimicrobial effect. *Vet. World*, 10,2: 170-175. https://doi.org/10.14202%2Fvetworld.2017.170-175

Wang, Y, Chang, Y, Yu, L, Zhang, C, Xu, X, Xue, Y, Xue, C, (2013). Crystalline structure and thermal property characterization of chitin from Antarctic krill (*Euphausia superba*). *Carbohydr. Polym.*, 92,1: 90-97. https://doi.org/10.1016/j.carbpol.2012.09.084

Zhao, X R, Xia, W S, (2006). Antimicrobial activities of chitosan and application in food preservation. *Chin. Food. Res. Dev.*, 27, 2: 157-160.