EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

MEDICAL ENTOMOLOGY & PARASITOLOGY

E

ISSN
2090-0783

WWW.EAJBS.EG.NET

Vol. 13 No. 1 (2021)

Citation: *Egypt. Acad. J. Biolog. Sci. (E-Medical Entom. & Parasitology Vol.13(1) pp **39-46**(2021)*
DOI: 10.21608/EAJBSE.2021.186760

Egypt. Acad. J. Biology. Sci., 13 (1):39–46 (2021)

Egyptian Academic Journal of Biological Sciences
E. Medical Entomology. & Parasitology

ISSN: 2090–0783
www.eajbse.journals.ekb.eg

Evaluation of the Antibacterial and Antifungal Activities of Chitosan Prepared from the American Cockroach, (*Periplaneta americana*)

Mahmoud T. Mahboub[1], Mostafa I. Hassan[1*], Ahmed S. Bream [1], Aly F. Mohamed[2] and Mohammad R. K. Abdel-Samad[1, 3]

1- Department of Zoology and Entomology, Faculty of Science, Al-Azhar University, Cairo, Egypt.
2- Research and Development Sector, VACSERA, Giza, Egypt.
3- Al-Azhar Technology Incubator, Al-Azhar University, Cairo, Egypt.
E-mail: mostafa012@gmail.com

ARTICLE INFO

Article History
Received:20/4/2021
Accepted:12/6/2021

Keywords:
Antibacterial, Antifungal, Chitosan, American cockroach.

ABSTRACT

Microorganisms such as bacteria and fungi are developing resistance to the current therapies easily and the presently available antibacterial, antifungal agents and pesticides are certainly very pricey and harmful. So, the necessary shift to use natural antimicrobial extract (*Periplaneta americana* chitosan) seems to be more effective, economic and safe to the public health. The American cockroach chitosan was dissolved in 1 % acetic acid and diluted to different concentrations then, seeded in 96 well tissue culture plates to evaluate the antibacterial activity by using MTT assay and the microbial growth was calculated by ELISA microplate reader. The American cockroach chitosan showed an equal minimum inhibitory concentration (MIC) antibacterial action against two (Gram-positive) bacterial strains *Staphylococcus aureus*, and *Bacillus subtilis*, recording MIC = 2000 µg/ml. Also, chitosan showed MIC antibacterial activity against *Escherichia coli* and *Salmonella typhimurim* (Gram-negative) bacteria recording MIC = 1000 µg/ml, and MIC = 2000 µg/ml respectively. While chitosan showed non-antifungal activity against *Candida albicans*. Natural chitosan could be considered as a determined factor affecting on the biological activities mentioned in this study.

INTRODUCTION

The increasing complication of antibiotic resistance by microbial organism's requests to research for new natural compounds, so insects have attracted the concern of entomologists for several reasons regarded as availability, widely and biodiversity of its species. The bioactive effect of some proteins that have been characterized from insects, with antimicrobial effects, were fascinating scientists for their potential benefits. However, recent improvement in biotechnology helped scientists to evaluate the antimicrobial activity of the insect's extracts (Ratcliffe *et al*., 2011). Cockroaches are capable of acquiring and infecting other cockroaches and objects, therefore implicating them as potential vectors of food borne pathogens in poultry production and processing facilities (Kopanic *et al.* 1994).

The insect immune responses based on the knowledge of the pathogen as nonself, farther on the induction of suitable genes and biochemical pathways appear in the manufacture of a potent arsenal of antibacterial ingredient (Seufi, *et al*., 2011).

Insects (cockroaches) protected themselves from pathogens and parasites via a powerful innate immune system, as cuticle immune, which contains antimicrobial agents (Pai *et al*. 2004). The chitin extracted from cuticle and chitosan of the cockroach (*Periplaneta americana*), are a versatile material with effectiveness in reducing microorganism growth and multiplication (Goy *et al*. 2009; Kim, *et al*. 2017). Insect extracts showed antimicrobial activities used universally in medicine for managing many diseases (Feng *et al*., 2009), also ingredients isolated from insects indicated antifungal activity against multi pathogenic fungi (Tomie *et al*., 2003). Chitosan, a natural antimicrobial agent, kept antimicrobial activity in different matrices and that provided a promising solution to enhance animal and public health (Ma, 2017). Chitosan obtained from chitin exhibited excellent antimicrobial activities against many species of Gram-positive and Gram-negative pathogenic bacteria (Chien *et al*., 2016). Chitosan was characterized by its highly microbicidal activities (Balicka-Ramisz *et al.*, 2005).

The purpose of this research was to investigate the antibacterial and antifungal activities of *Periplaneta americana* chitosan, against two (Gram-positive) bacteria namely, *Staphylococcus aureus* and *Bacillus subtilis*, and two different (Gram-negative) bacteria, *Salmonella typhimurim* and *Escherichia coli*, and a single fungal model, *Candida albican*s.

MATERIALS AND METHODS

1. The Antimicrobial Activity of Chitosan:

The American cockroach chitosan was prepared and characterized with a degree of deacetylation (DDA) above 50% acorrding to Mahmoud *et al*., (2021). Chitosan was dissolved in 1 % acetic acid (v/v) and diluted to a concentration of 8 mg/ml, with 1:2 serial dilutions to reach concentrations ranging from 8000 to 3.9 µg / ml. A quantity of 5 µl of each dilution was seeded in 96 well tissue culture plates, as well as a sterility control and growth control. Each test and growth control well was inoculated with 5 µl of microbial suspension (10^5 CFU/well).10 µl methyl-thiazolyl-tetrazolium (MTT) (Mosmann 1983) (5 mg/ml) was dispensed to each well and the plates were incubated for 3 h at 37 °C, 50 µl of the Dimethyl sulfoxide (DMSO) solution was added to these wells at room temperature for 30 minutes, then it was readied using ELISA microplate reader at 750 nm, then the microbial growth was calculated by equation.

$$\text{Microbial growth } \% = \frac{\text{Mean OD of treated test}}{\text{Mean OD of growth control}} \times 100$$

OD → Optical density.

2. The Antibacterial Activity:

The antibacterial activity was evaluated by the minimum inhibitory concentration (MIC) method, according to the method of Souza *et al*., (2005). All strains were tested and obtained from The Laboratory of Microbiology, Botany and Microbiology Department, Faculty of Science (Boys), Al-Azhar University, Nasr City, Cairo, Egypt. Four pathogenic bacterial strains were used for the antibacterial assay belonging to two groups:

- Gram-positive bacteria:

Staphylococcus aureus.
Bacillus subtilis

- Gram-negative bacteria:

Escherichia coli
Salmonella typhimurim

The microorganisms were growing in nutrient agar medium consisted of: peptone 5.0 gm/L; beef extract 3.0 gm/L; and agar-agar 15.0 gm/L the pH was adjusted to 7.0 before sterilization (Tadashi 1975).

````
````

3. The Antifungal Activity:

The antifungal activity was evaluated by the minimum inhibitory concentration (MIC) method, according to the method of Souza *et al*., (2005). The single fungal strain (*Candida albicans)* was obtained from The Laboratory of Microbiology, Botany and Microbiology Department, Faculty of Science (Boys), Al-Azhar University, Nasr City, Cairo, Egypt.

The microorganism was growing in a sucrose-nitrate agar medium consisted of sucrose 30 gm/L; $NaNO_3$ 2.0 gm/L; K_2HPO_4 1.0 gm/L; $MgSO_4$. $7H_2O$, 0.5 gm/L; agar 15.0 gm/L and distilled water 1000 ml. The pH value was adjusted at 7-7.3 before sterilization (Tadashi 1975).

RESULTS

1. Evaluation of the Antibacterial Activity of Chitosan:

I. The Antibacterial Activity of the Chitosan Against Gram-Positive Bacteria:

Chitosan with different concentrations was tested for its antibacterial activity, data arranged in table (1) by using the minimum inhibitory concentration (MIC) assay, and illustrated in figure (1). The antibacterial (growth- inhibitory) activity of chitosan after 24h (post-treatment) against *Staphylococcus aureus* showed complete inhibition percentage (100%) at concentrations of; 8000, 4000 and 2000µg/ml, and decreased gradually to the lowest concentrations until no inhibition recorded at a concentration of 31.25 µg/ml.

Also, chitosan showed 100% inhibition activity against *Bacillus subtilis* at concentrations of; 8000, 4000 and 2000µg/ml, while it decreased sequentially to the lowest concentrations until no inhibition recorded a concentration of 15.63µg/ml. From the results, it was obvious that chitosan recorded minimum inhibitory concentration antibacterial activity of 2000 µg/ml against the two tested Gram-positive bacterial strains.

Table 1: Evaluation of *P. americana* chitosan antibacterial activity against Gram-positive bacteria using the MIC assay.

Chitosan concentration µg/ml	*Staphylococcus aureus*	*Bacillus subtilis*
8000	100	100
4000	100	100
2000	100	100
1000	86.94	84.25
500	70.36	66.96
250	52.2	52.71
125	38.05	41.93
62.5	8.22	18.09
31.25	0	6.74
15.63	0	0
7.81	0	0
3.9	0	0

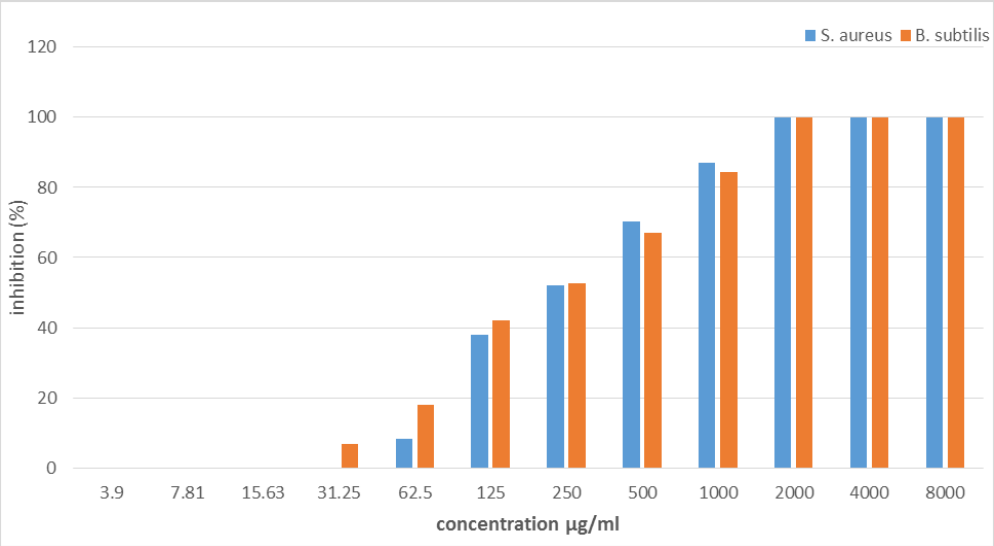

Fig.1: The antibacterial activity of *P. americana* chitosan against Gram- negative bacteria by using the MIC assay.

II. The Antibacterial Activity of Chitosan Against Gram-Negative Bacteria:

As shown in table (2) and figure (2) the antibacterial (growth- inhibitory) activity of chitosan after 24h post-treatment against *Escherichia coli* showed complete inhibition percentage (100 %) at concentrations of 8000, 4000, 2000 and 1000 µg/ml, and decreased gradually at the lowest concentrations until no inhibition recorded at concentration of 3.9 µg/ml.

On the other hand chitosan showed 100 % inhibition activity against *Salmonella typhimurim* at concentrations of; 8000, 4000 and 2000µg/ml, while it decreased sequentially to the lowest concentrations until no inhibition recorded at 7.81µg/ml. From the results, it was obvious that chitosan showed acceptable antibacterial activity against the two different (Gram-negative) bacterial strains. The minimum inhibitory concentrations were 1000 µg/ml and 2000 µg/ml for *Escherichia coli and Salmonella typhimurim*, respectively.

Table 2: Evaluation of the American Cockroach chitosan antibacterial activity against Gram-negative bacteria using the MIC assay.

Chitosan concentration µg/ml	*Escherichia coli*	*Salmonella typhimurim*
8000	100	100
4000	100	100
2000	100	100
1000	100	86.556
500	91.95	67.71
250	70.2	52.03
125	56.7	36.27
62.5	39.734	24.5
31.25	31.756	15.31
15.63	17.58	9.01
7.81	7.72	0
3.9	0	0

````
````

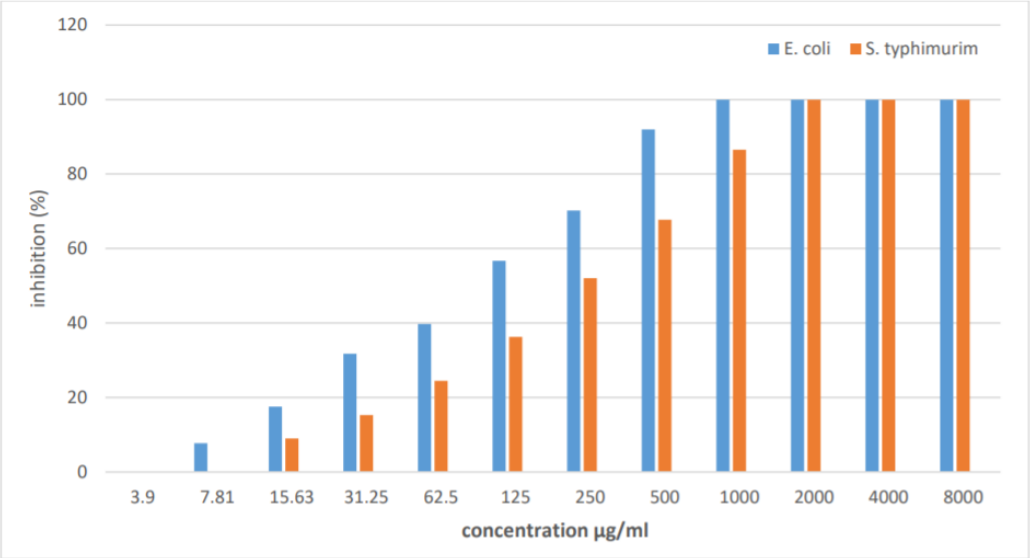

Fig.2: The antibacterial activity of American Cockroach chitosan against Gram-negative bacteria by using the MIC assay.

2. The Antifungal Activity of Chitosan*:*

Chitosan with different concentrations was tested for its antifungal activity against *Candida albicans,* by using the MIC assay. Non-antifungal (growth-inhibitory) activity of chitosan was recorded after 24h post-treatment.

DISCUSSION

In the present study, promising results of the American cockroach chitosan against *Staphylococcus aureus* (Gram-positive) bacteria were showed minimum inhibitory concentration in covenant with results of Gerasimenko *et al.,* (2004) where they found that bee chitosan also suppressed the growth of *S. aurous* at concentrations of 0.5 and 0.25%, likewise İlk *et al*., (2020) showed that (Gram-positive) bacteria *S. aurous* inhibited by chitosan membrane obtained from insect corneal lenses of *Tetanus bovines*. While on the conflict with our results, the chitosan synthesized from fish scales, crab and shrimp shells didn't show any activities against *S. aurous* according to Kumara *et al*., (2017).

The American cockroach chitosan showed decries in the bacterial growth of *Bacillus subtitles,* Chandrasekaran *et al*., (2020) and Tikhonov *et al*., (2006) results attained similar where they found that chitosan itself possesses antimicrobial activity against many Gram-positive bacteria as *B. subtilis.* In addition, No *et al.,* (2002) explored that chitosan with different molecular weights possesses antibacterial activity against Gram-positive bacteria (*Bacillus* sp*).*

Chitosan showed strong inhibitory activity against *Escherichia coli* after 24h post-treatment. In agreement with Divya *et al*., (2017) chitosan has antibacterial activity, against Gram-negative bacteria, *E. coli* with minimum inhibitory concentration (10% MIC). In addition, Fei Liu *et al*., (2001) observed an effective antibacterial activity of chitosan against *E. coli* with (20% MIC) depending on the molecular weight (MW) of chitosan. On the other hand, Shin, *et al*., (2019) disagreed with our results, where they found that chitosan from the mealworm beetle showed non-antibacterial activity against *E. coli* by using the MIC assay.

From results it was obvious that the American cockroach chitosan showed potent antibacterial activity against Gram-negative bacteria *Salmonella typhimurim,* in covenant with these results, (Tsai *et al*., 2002) illustrated that chitosan obtained from shrimp shell showed antibacterial action against *S. typhimurim*. Similarly with the results of Balicka-Ramisz *et al*., (2005) the effectiveness of crab chitosan solution against *Salmonella* sp, showed powerful,

effectiveness and inhibitory activity against the bacterial strain with 2 MIC.

On the other hand, our results showed non-antifungal activity against *Candida albicans*, hassling with these results Basseri *et al*., (2019) tested the antifungal activity of chitosan against *Candida albicans* where they noted that chitosan showed non-antifungal activities. In the opposite of Alburquenque *et al*., (2010) effect of chitosan against clinical *Candida* sp isolates exhibited significant antifungal activity.

In general, it was obvious that chitosan possesses antibacterial activity against tested bacteria, with no action against *Candida albicans*, in line with Shahraki *et al.,* (2018) extracted chitosan showed different degrees of antibacterial activity against both Gram-positive and Gram-negative bacteria such as *S. aureus, E. coli*, and *P. aeruginosa*. In addition, chitosan did not show any activity against *C. albicans* fungi.

CONCLUSION

Chitosan is a natural polysaccharide commonly found as a constituent of the cuticle of insects, as in the American cockroach. It has a biological role as an antibacterial and antifungal agent. The present results showed promising antibacterial activities of the American cockroach chitosan against the tested (Gram-positive) bacteria namely; *Staphylococcus aureus* and *Bacillus subtilis*, while chitosan showed reasonable antibacterial activities against both tested (Gram-negative) bacteria; *Escherichia coli* and *Salmonella typhimurim*. On the other hand, chitosan didn't show any antifungal activity against *Candida albicans*.

REFERENCES

Alburquenque, C.; Bucarey, S. A.; Neira-Carrillo, A.; Urzúa, B.; Hermosilla, G. and Tapia, C. V. (2010): Antifungal activity of low molecular weight chitosan against clinical isolates of *Candida spp*. *Medical Mycology*, *48*(8), 1018-1023.

Balicka-Ramisz, A.; Wojtasz-Pajak, A.; Pilarczyk, B.; Ramisz, A. and Laurans, L. (2005): Antibacterial and antifungal activity of chitosan. *International Society of Animal Hygiene*, 2, 406-408.

Basseri, H.; Bakhtiyari, R.; Hashemi, S. J.; Baniardelani, M.; Shahraki, H and Hosainpour, L. (2019): Antibacterial/antifungal activity of extracted chitosan from American cockroach (*Dictyoptera: Blattidae*) and German cockroach (*Blattodea: Blattellidae*). *Journal of Medical Entomology*, 56(5), 1208-1214.

Chandrasekaran, M.; Kim, K. D and Chun, S. C. (2020): Antibacterial activity of chitosan nanoparticles: *A review. Processes*, 8(9), 67-73.

Chien, R. C.; Yen, M. T and Mau, J. L. (2016): Antimicrobial and antitumor activities of chitosan from shiitake stipes, compared to commercial chitosan from crab shells. *Carbohydrate Polymers*, 138, 259–264.

Divya, K.; Vijayan, S.; George, T. K. and Jisha, M. S. (2017): Antimicrobial properties of chitosan nanoparticles: Mode of action and factors affecting activity. *Fibers and Polymers*, 18(2), 221-230.

Fei Liu, X.; Lin Guan, Y.; Zhi Yang, D.; Li, Z. and De Yao, K. (2001): Antibacterial action of chitosan and carboxymethylated chitosan. *Journal of Applied Polymer Science*, 79(7), 1324-1335.

Feng, Y.; Siu, K.; Wang, N.; Ng, K. M.; Tsao, S. W.; Nagamatsu, T. and Tong, Y. (2009): Bear bile: dilemma of traditional medicinal use and animal protection. *Journal of Ethnobiology and Ethnomedicine*, 5(1), 1-9.

Gerasimenko DV, Avdienko ID, Bannikova GE, Zueva OY and Varlamov VP. (2004): Antibacterial effects of water-soluble low-molecular weight chitosans on different microorganisms. *Applied*

Biochemistry and Microbiology, 40(8), 253-257.

Goy, R. C.; Britto, D. D. and Assis, O. B. (2009): A review of the antimicrobial activity of chitosan. *Polymers*, 19(3), 241-247.

İlk, S.; Ramanauskaitė, A.; Bilican, B. K.; Mulerčikas, P.; Cam, D.; Onses, M. S. and Kaya, M. (2020): Usage of natural chitosan membrane obtained from insect corneal lenses as a drug carrier and its potential for point of care tests. *Materials Science and Engineering*, 9(7), 110-112.

Kim, M. W.; Song, Y. S.; Seo, D. J.; Han, Y. S.; Jo, Y. H.; Noh, M. Y and Jung, W. (2017): Extraction of chitin and chitosan from the exoskeleton of the cockroach (*Periplaneta americana L.*). *Journal of Chitin and Chitosan*, 22(2), 76-81.

Kopanic Jr, R. J.; Sheldon, B. W and Wright, C. G. (1994): Cockroaches as vectors of Salmonella: laboratory and field trials. *Journal of food protection*, 57(2), 125-135.

Kumari, S.; Annamareddy, S. H. K.; Abanti, S. and Rath, P. K. (2017): Physicochemical properties and characterization of chitosan synthesized from fish scales, crab and shrimp shells. *International Journal of Biological Macromolecules*, 104, 1697–1705.

Ma, Z.; Garrido-Maestu, A. and Jeong, K. C. (2017): Application, mode of action, and in vivo activity of chitosan and its micro-and nanoparticles as antimicrobial agents: A review. *Carbohydrate Polymers*, 176, 257-265.

Mahmoud, T. Mahboub.; Mostafa, I. Hassan.; Ahmed, S. Bream.; Aly, F. Mohamed and Mohammad R. K. Abdel-Samad (2021): Preparation, Characterization and Anticancer Activity of Chitosan Prepared from the American Cockroach, *Periplaneta americana*. *Egyptian Academic Journal of Biological Sciences A. Entomology*, 14(2) 163-171

Mosmann, T. (1983): Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65(1-2), 55-63.

No, H. K.; Park, N. Y.; Lee, S. H. and Meyers, S. P. (2002): Antibacterial activity of chitosan and chitosan oligomers with different molecular weights. *International Journal of Food Microbiology*, 74(1-2), 65-72.

Pai, H. H.; Chen, W. C. and Peng, C. F. (2004): Cockroaches as potential vectors of nosocomial infections. *Infection Control and Hospital Epidemiology*, 25(11), 979-984.

Ratcliffe, N. A.; Mello, C. B.; Garcia, E. S.; Butt, T. M. and Azambuja, P. (2011): Insect natural products and processes: new treatments for human disease. *Insect Biochemistry and Molecular Biology*, 41(10), 747-769.

Seufi, A. M.; Hafez, E. E and Galal, F. H. (2011): Identification, phylogenetic analysis and expression profile of an anionic insect defensin gene, with antibacterial activity, from bacterial-challenged cotton leafworm, *Spodoptera littoralis*. *BioMed Central molecular biology*, 12(1), 1-14.

Shahraki, H.; Basseri, H. R.; Mirahmadi, H.; Bafghi, M. F.; Mehravarn, A.; Heydarian, P. and Esboei, B. R. (2018): Evaluation of antibacterial and antifungal activity of chitosan in integument of cockroaches. *International Journal of Basic Science in Medicine*, 3(3), 104-108.

Shin, C. S.; Kim, D. Y and Shin, W. S. (2019): Characterization of chitosan extracted from Mealworm Beetle (*Tenebrio molitor*, *Zophobas morio*) and Rhinoceros Beetle (*Allomyrina dichotoma*) and their antibacterial activities. *International Journal of*

Biological Macromolecules, 125, 72-77.

Souza, S. M. D.; Monache, F. D., and Smânia, A. (2005): Antibacterial activity of coumarins. *Zeitschrift fuer Naturforschung*, 60 (9-10), 693-700.

Tadashi, A. (1975): Culture media for actinomycetes. *The Society for Actinomycetes, Japan National Agricultural Library*. 1: 1-31.

Tikhonov, V. E.; Stepnova, E. A.; Babak, V. G.; Yamskov, I. A.; Palma-Guerrero, J.; Jansson, H. B. and Varlamov, V. P. (2006): Bactericidal and antifungal activities of a low molecular weight chitosan and its N-/2 (3) -(dodec-2-enyl) succinoyl/-derivatives. *Carbohydrate polymers*, 64(1), 66-72.

Tomie, T.; Ishibashi, J.; Furukawa, S.; Kobayashi, S.; Sawahata, R.; Asaoka, A and Yamakawa, M. (2003): Scarabaecin, a novel cysteine-containing antifungal peptide from the rhinoceros beetle, *Oryctes rhinoceros*. *Biochemical and Biophysical Research Communications*, 307(2), 261-266.

Tsai, G. J.; Zhang, S. L. and Shieh, P. L. (2004): Antimicrobial activity of a low-molecular-weight chitosan obtained from cellulase digestion of chitosan. *Journal of Food Protection*, 67(2), 396-398.

````
````