ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

The Antibacterial Activity of Chitosan between Different Extraction Method

Parisa Sadighara[1], Ahmad Erfanmanesh[2], Ehsan Haghi[1], Donya Nikaein[2], Tahereh Mohajerfar[2], Taha Tohidimoghadam[1], Mahmoud Bahmani[3], Abolfazl Abaszadeh[4], Mahmoud Rafieian-Kopaei[5*]

[1]Academic Center of Education, Culture and Research (ACECR), Tehran, Iran
[2]Department of Environmental Health, Food Safety Division, Faculty of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[3]Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran
[4]Department of Surgery, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran
[5]Medical Plants Research Center, Shahrekord University of Medical sciences, Shahrekord, Iran

***Corresponding author: E-Mail: rafieian@yahoo.com**

ABSTRACT

This survey described the relation between chitosan extraction methods and the antibacterial activity. Chitosan were extracted from shrimp waste according to the conventional method. But in step of deprotenisation, three process acid, alkaline, and enzyme extraction was used. The extracted chitosan evaluated by inhibition of bacterial growth against current foodborne bacteria. Therefore, Escherichia coli and Staphylococcus aureus are used to study the antimicrobial activity. These results demonstrated that good results can be achieved by enzymatic and alkaline treatment. Subject to economic advantages, alkaline can replace the other methods.

KEY WORDS: Shrimp Waste, Extraction Methods, Chitosan, Antimicrobial Activity.

1. INTRODUCTION

Infectious diseases still cause outbreaks of diseases which is cause economic disadvantage to people (Soroush, 2010; Taherikalani, 2008; Haghi-Ashteiani, 2007; Nakhjavani, 2013; Jabalameli, 2012; Khoramrooz, 2012; Shahsavan, 2012; Asadollahi, 2011; Sahebekhtiari, 2011; Shahsavan, 2011; Mahdi, 2007). About 50 percent of total body weight of shrimp is waste that is produced as a byproduct of the shrimp industry (Cahu, 2012). This waste can be used as a source for the extraction of chitin. Chitosan due to its non-toxicity and special properties has many applications.

The chitosan is used in food stuff, agriculture, cosmetic, pharmaceutical industries and wastewater treatment unit. Furthermore, chitosan has also shown the medicinal properties, such as cholesterol-lowering properties, drug delivery as well as anti-microbial properties (Puvvada, 2012). *Staphylococcus aureus* is a facultative microaerophilic gram-positive coccal bacterium. S. aureus is found on the skin, nose and throat of most people. Infected wounds and acne are rich sources (Ganguly, 2012). It can cause a range of illnesses, such as skin infections, pimples, impetigo, scalded skin syndrome, and life-threatening diseases, such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, sepsis, and food poisoning (McClements and Decker, 2000). Escherichia coli (E. coli) are bacteria found in foods, and intestines of people and animals. It can make to cause severe stomach cramps, diarrhea and vomiting. E. coli O157:H7 can induce kidney failure.

Antimicrobial activity is one of the attractive features of chitosan. The degree of this activity depends on the methods involved in production of chitosan (Tin, 2009).This paper has been designed with evaluating the effect of extraction method on the antibacterial activity of chitosan against these bacteria.

2. MATERIALS AND METHOD

The shrimp wastes, *Penaeus semisulcatus*, were collected from the processing plants. Then, the wastes were air dried in the shade and powdered. The experimental design 5 g of sample was placed in a test tube and dissolved in 1 N HCL for 24h at 28 ºC for demineralisation treatment.

In deproteinization step, three methods were used. The residues were divided to three parts and placed in 1 N NaOH, Trypsin and Trichloroacetic acid (TCA) respectively. In enzymatic method; 10% of trypsin was added to waste. In these step complex protein-carotenoids separated.

Then, the hydrolysate was centrifuged and the supernatant used for determination of total carotenoids. The remaining of this process is chitin. Chitin changes to chitosan in deacetylation process. In this process, the acetyl groups were removed from the chitin. For this purpose, the residue is placed in 50% NaOH and boiled at 100 °C for 2 hours (sadighara, 2016).

Examining the antimicrobial activities of chitosan Bacterial strains of Staphylococcus aureus29213 ATCC and Escherichia coli 35218 ATCC, was taken from Veterinary Tehran University.

Disk diffusion method: Disk diffusion method was used to survey the sensitivity of microbes to chitosan. First, some amount of fresh culture of standard bacteria was dissolved in the physiology serum until reaching the half McFarland turbidity then the suspension was cultured by using a sterile swab on surface of the medium (Muller Hinton) completely and in several directions.

20 micro liters of chitosan solution from different concentration (0.01, 0.1 and 1%) was injected into sterile discs and after being dried placed on the medium to form the halo. Discs containing antibiotics (Oxytetracycline, Penicillin and Streptomycin) were used as controls. Chitosan is soluble in acetic acid; therefore %1 acetic acid was also used as control.

Measuring of minimum inhibitory concentration (MIC) of chitosan: The lowest concentration of the antimicrobial agent that inhibits the growth of the microorganism was measured. It was detected by lack of visual turbidity, matching with a negative control (Tin, 2009).

In short, µl100 Mueller Hinton medium Broth was transferred to the plate 96 wells. Then, the serial of chitosan double dilution was prepared and added to the wells 1 to 10 of the plates. 100 µl bacterial suspensions added to wells 1 to 11. The well 11 was determined as a positive control while well 12 was selected as negative control.

The dilutions of antibiotics Serial was considered as a control drug and chitosan without bacteria was considered as chitosan control. All tests were performed in 3 replications. Plates were incubated at a temperature 28 ºC for 24 hours. To obtain a MIC, the optical absorption of plates was read by ELISA reader at a wavelength of nm620 at zero hour for duration of 24 hours after incubation.

3. RESULTS

Disk diffusion method: We determined the antimicrobial power of the extracted chitosans using two methods.The antimicrobial properties of extracted chitosans against Staphylococcus aurous and E. coli is shown in Table 1.

The results showed that chitosans extracted by enzymatic and alkaline method are effective antimicrobial agent against E. coli when compared with antibiotics. For E. coli the most active agents were oxyteracycline (36mm) followed by chitosan extracted by enzyme (19mm) and chitosan extracted by alkaline method (17mm). The sensitivity of Staphylococcus aurous to chitosan was more than E. coli.

Table.1. Diameter of non-growth halo (mm)

	Chitosan extracted by trypsin			**Chitosan extracted by TCA**			**Chitosan extracted by NaOH**			**oxytetracycline**	**Penicilline**	**streptomycin**	**Acetic acid 1%**
	%1	% 0.1	%0.01	%1	%0.1	%0.01	%1	%0.1	%0.01				
Halo Diameter *S.aureus*	23	15	0	19	13	0	25	16	0	29	27	7	0
Halo Diameter *E.coli*	19	10	6	14	9	0	17	11	0	36	8	13	0

Measuring of minimum inhibitory concentration (MIC) of chitosan: Measuring of MIC Results showed the antimicrobial activity of 0.025 diluted against all the evaluated bacteria. Then, reading of plates by ELISA reader confirmed almost the visual our observations. The result by ELISA reader was 0.025 diluted against E. coli and Staphylococcus aurous 0.0125.

DISCUSSION

In this study, antibacterial properties of chitosan were considered. Resulted achieved from this study showed the chitosan extracted by enzymatic and alkaline condition inhibits growth of the standard culture of bacteria. One of the issues that we face today is the increase of antibiotic-resistance of pathogenic bacteria (Gangoue, 2006). Improving the effectiveness and decreasing the toxicity of antibiotics are the two basic objectives in the development of replaced antimicrobial agents (Tin, 2009).

Furthermore, everyday are growing tendency to use synthetic chemical-free food. Synthetic antimicrobials have been used as preservatives to control microbial risks. Nevertheless, these compounds are not entirely satisfactory for consumers seeking natural and healthy foods (Hernandez-Ochoaa, 2012).

Chitosan as a natural polysaccharide can be considered as an appropriate antimicrobial preservative in the food and medicine industries (Rabea, 2009). The pervious study suggest on the useing of chitosan in combination with antibiotics in pharmaceutical preparations (Tin, 2009). Chitosan due to positive amine groups with the combined bacterial cell membrane causes disrupt in material transfer and reducing their growth, the type of environment or the food chitosan present in it is also effective in the antimicrobial activities of it; in a manner that chitosan is more effective in the foods with a lower PH because an increasing number of groups charged with amine acid (+ NH3) are present in acidic PH (Crini, 2005).

CONCLUSION

According to the previous study chitosan extracted by different methods have some differences in the degree of de-acetylation and the size of decomposition of proteins and the level/degree of purity (Sadighara, 2015). The observed higher levels of inhibition of growth could be attributed to its produce method. Our data showed that the most effective method is enzymatic method and followed by alkaline method in antibacterial activity.

The result of present study showed the produce methods could have an impact on its antimicrobial properties.

REFERENCES

Asadollahi K, Taherikalani M, Maleki A, Asadollahi P, Emaneini M, Diversity of aminoglycoside modifying enzyme genes among multidrug resistant Acinetobacter baumannii genotypes isolated from nosocomial infections in Tehran hospitals and their association with class 1 integrons, 58(4), 2011, 359-370.

Cahu T, Santos S, Mendes A, Cordula C, Chavante S, Carvalho L, Recovery of protein, chitin, carotenoids and glycosaminoglycans from Pacific white shrimp (Litopenaeus vannamei) processing waste. Process Biochemistry, 47, 2012, 570-577.

Crini G, Recent developments in polysaccharide-based materials used as adsorbents in wastewater treatment. Progress in Polymer Science, 30, 2005, 38–70.

Gangoue PJ, Koulla SHS, Ngassam P, Adiogo D, Ndumbe P, Antimicrobial activity against gram negative bacilli from Yaounde Central Hospital, Cameroon. Afr Health Sci, 6, 2006, 232-235.

Ganguly S, Mukhopadhayay SK, Subhasish Biswas S,Potential threat to human health from foodborne illness having serious implications on public health- a Review, Int J Chem Biochem Sci, 10, 2012, 65–68.

Haghi-Ashteiani M, Sadeghifard N, Abedini M, Soroush S, Taheri-Kalani M, Etiology and antibacterial resistance of bacterial urinary tract infections in children's medical center, Tehran, Iran, Acta Medica Iranica, 45 (2), 2007, 153-157.

Hernandez-Ochoaa L, Macıas-Castan edaa CA, Nevarez-Moorillo GV, Salas-Munoza E, Sandoval-Salasb F, Antimicrobial activity of chitosan-based films including spices' essential oils and functional extracts, CyTA – Journal of Food, 10, 2012, 85–91.

Jabalameli F, Mirsalehian A, Khoramian B, Taherikalani M, Emaneini M, Evaluation of biofilm production and characterization of genes encoding type III secretion system among Pseudomonas aeruginosa isolated from burn patients, Burns, 38 (8), 2012, 1192-1197.

Khoramrooz SS, Mirsalehian A, Emaneini M, Borghaei P, Razmpa E, Frequency of Alloicoccus otitidis, Streptococcus pneumoniae, Moraxella catarrhalis and Haemophilus influenzae in children with otitis media with effusion (OME) in Iranian patients. Auris Nasus Larynx, 39 (4), 2012, 369-373.

Mahdi A, Mohammad N, Morovat T, Abbas M, Ali H, Rapid identification of iranian acinetobacter baumannii strains by single PCR assay using BLA oxa-51-like carbapenemase and evaluation of the antimicrobial resistance profiles of the isolates, Acta Microbiologica et Immunologica Hungarica, 57 (2), 2007, 87-94.

McClements DJ, Decker EA, Lipid peroxidation in Oil - in - water emulsion, impact of molecular environment on chemical reaction in heterogeneous food systems. J food sci, 65, 2002, 1270–1282.

Nakhjavani F.A, Emaneini M, Hosseini H, Taherikalani M, Mirsalehian A, Molecular analysis of typical and atypical enteropathogenic Escherichia coli (EPEC) isolated from children with diarrhoea. Journal of Medical Microbiology, 62 (2), 2013, 191-195.

Puvvada YS, Vankayalapti S, Sukhavasi S, Extraction of chitin from chitosan from exoskeleton of shrimp for application in the pharmaceutical industry. International Current Pharmaceutical Journal, 1, 2012, 258-263.

Rabea EI, Badawy MET, Stevens CV, Smagghe G, Steurbaut V,Chitosan as antimicrobial agent, Applications and mode of action. Biomacromolec, 6, 2003, 1457–1465.

Sadighara P, Tohidi Moghadam H, Eskandari S, Salehi A, Optimization of extraction of chitosan and carotenoids from shrimp waste. International Jornal of Fisheries and Aquatic studies, 2, 2015, 51-54.

Sahebekhtiari N, Nochi Z, Amin Eslampour M, Reza Zali M, Emaneini M, Characterization of staphylococcus aureus strains isolated from raw milk of bovine subclinical mastitis in tehran and mashhad, Acta Microbiol Immunol Hung, 58(2), 2011, 113-121.

Shahsavan S, Emaneini M, Noorazar Khoshgnab B, Eslampour M.A, Taherikalani M, A high prevalence of mupirocin and macrolide resistance determinant among Staphylococcus aureus strains isolated from burnt patients. Burns, 38 (3), 2012, 378-382.

Shahsavan S, Jabalameli L, Maleknejad P, Feizabadi M, Emaneini M, Molecular analysis and antimicrobial susceptibility of methicillin resistant Staphylococcus aureus in one of the hospitals of Tehran University of Medical Sciences, High prevalence of sequence type 239 (ST239) clone, Acta Microbiologica et Immunologica Hungarica, 58(1), 2011, 31-39.

Soroush S, Haghi-Ashtiani MT, Taheri-Kalani M, Yasemi M, Paiman H, Antimicrobial resistance of nosocomial strain of Acinetobacter baumannii in children's medical center of Tehran, A 6-year prospective study, Acta Medica Iranica, 48 (3), 2010, 178-184.

Taherikalani M, Etemadi G, Geliani KN, Soroush S, Feizabadi M.M, Emergence of multi and pan-drug resistance Acinetobacter baumannii carrying blaOXA-type-carbapenemase genes among burn patients in Tehran, Iran, Saudi Medical Journal, 29(4), 2008, 623-624.

Tin S, Sakharkar KR, Lim CS, Sakharkar MK, Activity of Chitosans in combination with antibiotics in Pseudomonas Aeruginosa, Int.J.Biol.Sci, 5, 2009, 153-160.