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RESEARCH ARTICLE

ISOLATION AND CHARACTERIZATION OF CHITIN FROM PRAWN SHELL WASTE AND INCORPORATION INTO MEDICAL TEXTILES

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ABSTRACT

Chitin is one of the most abundant natural polysaccharide on earth. Different crustaceans, molluses, marine diatoms, insects, algae, fungi and yeast synthesize it. According to the amount of chitin produced annually over the world, it is second abundant polymer next to cellulose. Recent investigations confirm the suitability of chitin and its derivatives in chemistry, biotechnology, medicine, and food processing, environmental protection and also used as a safe excipient in drug delivery formulations. Our present study focused that on the isolate the prawn shell waste and conversion to chitosan.Chitosan was characterized using IR spectrum and compared with standard to confirm the presence of chitosan in the shell of prawn. We attempted to incorporate latex milk of *Euphorbia neriifolia*, which has potent medicinal properties with chitosan and check its antimicrobial activity. The results of the present study proved the antimicrobial activity exhibited by the samples incorporated in medical textiles on test organisms.

INTRODUCTION

Chitin is one of the most abundant natural polysaccharides produced by many living organisms (Alam *et al*., 2008) It has a crystalline structure and it constitutes a network of organized fibers. Chitin is versatile and environmental friendly. Its derivatives have been used in virtually every significant segment of the economy. Both chitin and chitosan show very good compatibility but this property depends on the characteristics of the sample (natural source, method of preparation).The cytocompatibility of chitosan has been proved *in vitro* with myocardial endothelial and epithelial cells, fibroblast, hepatocytes, chondrocytes and keratinocytes (Aranaz *et al*.,2009)

Euphorbia neriifolia is a large and widely distributed genus with species containing white, milky latex in vacuoles of specialized secretory cells called laticifers. Lattices of many *Euphorbia* species have been used as purgatives. (Lukovic *et al*., 2009). The latex of *Euphorbia neriifolia* facilitated the wound healing process as evidenced by increase in tensile strength, DNA content, epithelization and angiogenesis. (Gaur *et al.*, 2009)

Since chitin is biocompatible and studies have shown that it aids in slow drug release, we attempted to exploit this property of chitin.

METHODOLOGY

Isolation of chitin and chitosan preparation

The prawn fish shell waste was collected thrice a week from the Ukkadam fish market in Coimbatore.The prawn fish shell waste were washed thoroughly with running water for two or three times to remove the soil particles, soluble organics, adherent protein and other impurities and dried in a forced air oven at 60°C for 24 hours. Chitin was extracted from the prawn by deproteinization and demineralization.Chitosan was preparedfrom the chitin through deacetylation process following the method of Hong 1989.

CHARACTERIZATION OF CHITOSAN

FT-IR spectroscopy of solid samples of chitin from prawn shell relied on a Bio-Rad FTIS-40 (model USA).Sample(10µg) was mixed with 100µg of dried potassium bromide (KBr) and compressed to prepare a salt disc (10mm diameter) for reading the spectrum further. The structure of isolated chitin and chitosan was confirmed by doing FT – IR spectral analysis and compared with standard chitosan.

PREPARATION OF *Euphorbia neriifolia* MILK:

Euphorbia neriifolia latex milk was taken and it was extracted with methanol. The mixture was evaporated at 60°Cand then residue was dissolved in 500µl of DMSO.

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ANTIMICROBIAL ACTIVITY

Antibacterial sensitivity test

Antibacterial assay was carried out by Agar well and Disc diffusion method using bacteria such as *Salmonella typhi, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* by the method of NCCLS., 1997.

Antifungal sensitivity test

Antifungal assay was carried out by Spore germination assay using fungi such as *Aspergillus flavus,Aspergillus fumigatus,* and *Mucor* as outlined by Schlubaum 1986.

Antimicrobial assays were done and minimum inhibitory concentration was determined.

RESULTS

Chitin was isolated from prawn shell waste. Deproteinization and demineralization are the important step in chitin purification.Chitin thus isolated was converted to chitosan by deacetylation.

Characterization of chitosan

The IR spectrum of the standard chitosan contained 15 major peaks (Fig.1) whereas the IR spectrum of the sample from prawn waste (Fig. 2) also recorded 13 peaks.

Antibiotic sensitivity test (AST)

The sensitivity levels of the microorganisms *Salmonella typhi, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae* to standard antibiotics ampicilin, erythromycin, gentamycin, and streptomycin in terms of growth inhibition zone.

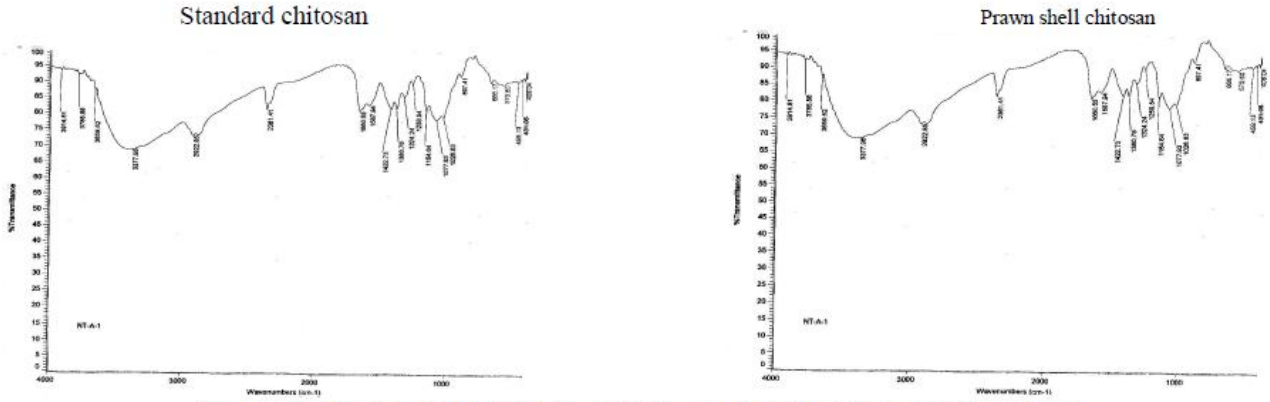

Fig I: Characterization of isolated chitosan and standard chitosan by FT-IR spectrum

The results obtained Showed that the pathogens are not susceptible to treatment with these common antibiotics hence the need to try alternative source to kill them

ANTIBACTERIAL ACTIVITY

Results presented in Table 1 shows that chitosan with milk was found to be effective against the tested bacterial species. It is moderately effective against *Pseudomonas aeruginosa*. Latex milk was less effective against *Salmonella typhi, Pseudomonas aeruginosa* and *Klebsiella pneumonia.*Among the three samples combined effect of both was found to be effective in action showing maximum zone of inhibition followed by chitosan and milk. Methanol was also tested because it was used for preparation of latex milk extract. Methanol by itself showed no zone of inhibition showing that it has no antibacterial effect.

Antibacterial activity was assessed by yet another method namely disc diffusion method and results are presented. Table III indicates the antibacterial activity of chitosan, latex milk and combination of chitosan and latex milk by disc diffusion method.

Chitosan and chitosan+milk were found to be effective against the tested bacterial species.Chitosan was found to be more effective against *Klebsiella pneumoniae.*It is moderately active against *Pseudomonas aeruginosa* and *Salmonella typhi.*Chitosan with milk were found to be more effective against *Pseudomonas aeruginosa* and it is moderately effective against *Salmonella typhi* and *Klebsiella pneumoniae.*Latex milk was less effective against *Salmonella typhi, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.*Among the three samples chitosan and chitosan with latex milk was found to be effective in action, showing maximum zone of inhibition followed by latex milk. The results of antibacterial activity assessed by both the methods showed that chitosan and latex milk together possess better antibacterial activity compared to either of them alone. This supports the fact that it can be used for preparing antibacterial compounds. Several studies support our findings where chitosan and various plant extracts are used as antibacterial agents.

Plate I indicates the antibacterial activity of chitosan, latex milk and combination of chitosan and latex milk incorporated in band aid cloth by diffusion method.

Chitosan and chitosan + milk were found to be effective against the tested bacterial species.Chitosan was found to be more effective against *Klebsiella pneumoniae* and *Salmonella typhi*. Hence it was used to again check the zone of inhibition to various microorganisms by incorporation of chitosan+milk at varying concentrations and measuring the zone of inhibition. The minimum inhibitory concentration was determined.

Plate 1 Antibacterial Activity

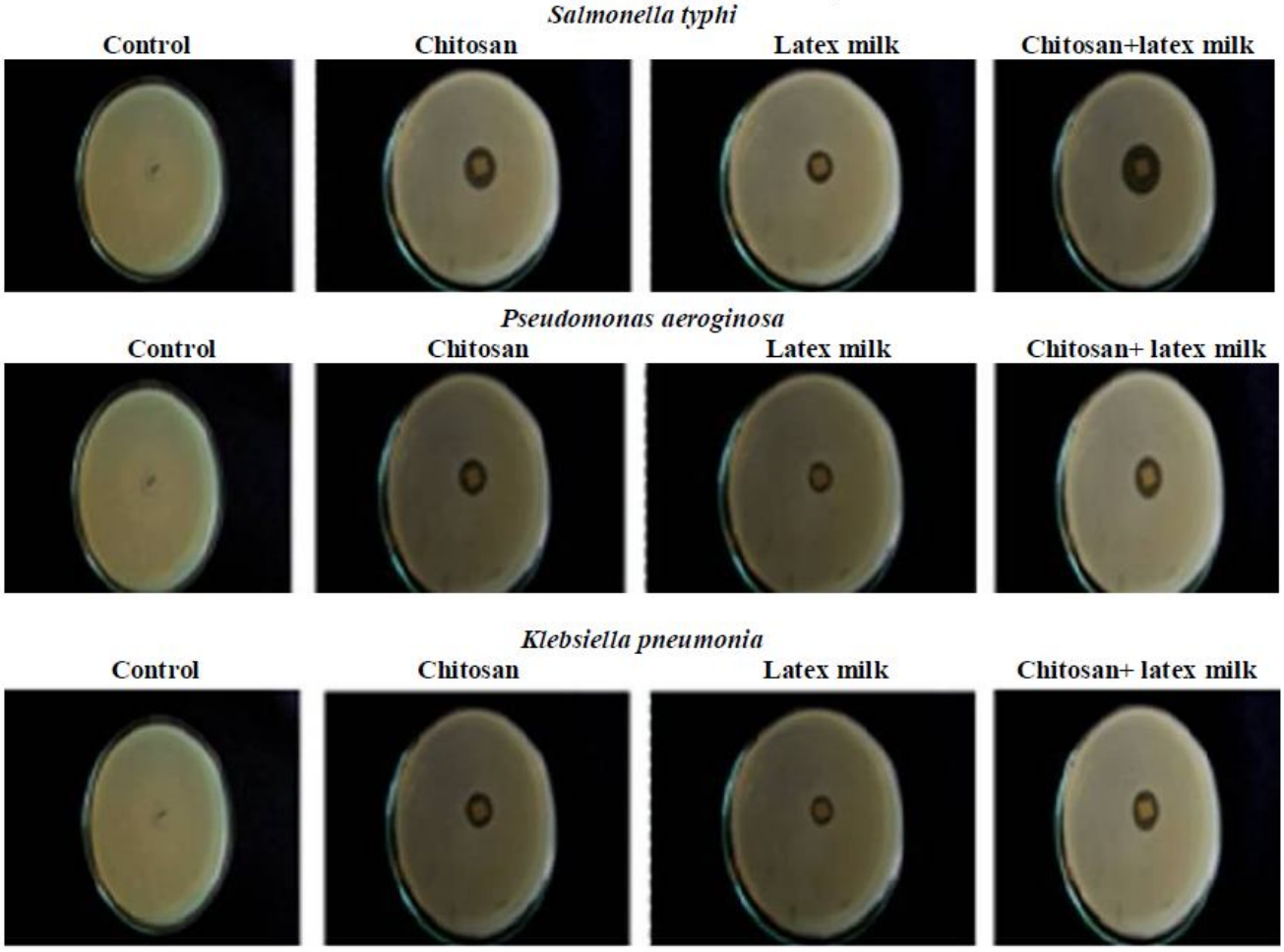

Table 1 Antibacterial activity by agar well diffusion method

S.no	Microorganisms	Diameter of zone of inhibition (mm)			
		Chitosan	Latex milk	Chitosan + Milk	Methanol
1.	*Salmonella typhi*	7	5	8	NI
2.	*Pseudomonas aeroginosa*	6	5	7	NI
3.	*Klebsiella pneumoniae*	7	5	8	3

NI –No inhibition

Table 2 Antibacterial activity by disc diffusion method

S.no	Microorganisms	Diameter of zone of inhibition (mm)			
		Chitosan	Latex milk	Chitosan + Milk	Methanol
1.	*Salmonella typhi*	5	4	5	NI
2.	*Pseudomonas aeroginosa*	5	4	7	NI
3.	*Klebsiella pneumoniae*	7	4	5	3

NI –No inhibition

Table 3 Minimal inhibitory concentrations

	Salmonella typhi(µg/100µl)							*Pseudomonas aeroginosa(µg/100µ)*							*Klebsiella pneumonia(µg/100µl)*						
amples	0	100	50	25	12	6	3	0	100	50	25	12	6	3	0	100	50	25	12	6	3
Chit0san+Milk	-	+	+	+	+	-	-	-	+	+	+	+	-	-	-	+	+	+	+	+	-
Chit0san+Milk	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+	+	+	-	-	-
Chit0san+Milk	-	+	+	+	+	+	-	-	+	+	+	+	-	-	-	+	+	+	-	-	-

Table 4 Spore Germination Assay

S.No	Microorganisms	Samples			
		Chitosan	Latex milk	Chitosan + Milk	Control
1.	*Aspergillus flavus*	+	+	+	–
2.	*Aspergillus fumigatus*	+	+	+	–
3.	*Mucor*	+	+	+	–

+ = Inhibition of spore germination
- = No inhibition of spore germination

Antibacterial activity of varying concentrations of chitosan and or latex milk

Fig. 1 indicates the antibacterial activity of chitosan, latex milk and chitosan with latex milk at different concentrations by incorporation in band aid cloth which was more effective in executing the antibacterial effect compared to the other methods.

Chitosan at 60µg, milk at 20mg and chitosan+latex milk at 60µl concentrations were found to be more effective against *Salmonella typhi, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.*Chitosan at 50µg, milk at 15 mg and chitosan + latex milk at 50µl concentration were found to be moderately effective against *Salmonella typhi, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.*Lower concentrations of all the three were found to be less effective against the microorganisms tested. Among the three samples at four different concentrations, higher concentration of chitosan was found to be more effective, showing maximum zone of inhibition followed by higher concentration of latex milk and chitosan + latex milk.

ANTIFUNGAL ACTIVITY

Table VI indicates the antifungal activity of chitosan, latex milk and combined effect of both by spore germination assay.

The percentage of spore germination was drastically reduced in *Aspergillus flavus, Aspergillus fumigatus* and *Muco*r. Finaly three samples were incorporated into the fabric implicating its use to be exploited in medical textiles.

DISCUSSION

Chitin was converted to chitosan which was characterized by FT- IR spectrum.Water-soluble chitosan from shrimp shell showed better antibacterial activity than crude chitosan without degradation treatment. This indicated the high potential of water-soluble chitosan as a antibacterial agent (Du *et al.,* 2009). Chitosan has been observed to act more quickly on bacteria, and activity against typhoid organisms are comparable to the standard antibiotics used in clinical practice. The effectiveness of chitosan varies and is dependent on species of target microorganisms (Goy *et al.,* 2009). Marques *et al.,* (2008) have shown that chitosan exhibited strong antibacterial activities at low temperature followed by oregano and garlic against *S.enterica.*

The present study too chitosan along with latex milk was effective against the bacterial species tested. Several plant extracts possess antifungal effect like *Euphorbia neriifolia.*

Awadalla and Mahmoud, (2005) reported that chitosan, was effective against *Alternaria alternata* and *Fusarium.sp.lycopersici,* which causes black mould of tomatoes. Antifungal activity of chitosan was reported against *Fusarium dimerum pezig, Aspergillus nidulans wint, Aspergillus fumigatus fresenius* and *Aspergillus japonicus saito* that occurred in mango and dragen fruits.The methanolic extract of *Caesalpinia pulcherrima* and *Cyperus rotundus* showed best antifungal activity against *A.candidus. Saussurea lappa* showed best antifungal activity against *Aspergillus flavus* followed by *Trapanatans* and *Mangifera indica* (Parekh and Chanda, 2008).

The three extracts of *Calotropis procera* namely, methanol, ethyl acetate and water have exhibited antifungal activity against *Fusarium* and *Trochoporium vesiculosum* (Devi *et al.,* 2008).Chlorhexidine exhibits broad-spectrum antifungal activity against *Candida albicans.* (Rathore *et al.,* 2009).These studies support our results were plant extracts can be exploited for their antimicrobial effect.

CONCLUSION

Thus chitin isolated from prawn shell waste was comparable with that of standard chitosan as revealed by the results of IR spectrum. Both were effective in controlling the growth of bacterial and fungal species tested. The chitosan and latex milk were effective in controlling the growth of bacteria and fungi. We attempted to incorporate chitosan in band-aid cloth by exhaust method. Chitosan got incorporated in the band aid cloth suggesting its use in production of medical textiles.

Since the combined effect of chitosan and latex milk was effective, it can be exploited in commercial preparations of textiles with antimicrobial finish and chitosan-based dressings.

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References

Alam,R., Khan,M.A., Khan,R.A., Ghoshal,S and Mondal,M.I.H.(2008), Study on the physico-mechanical properties of photo-cured chitosan films with oligomer and acrylate monomer, J. Polym. Environ, 16,213-219.

Aranaz,I., Mengibar,M., Harris,R., Panos,I., Mirlles,B., Acosta,N., Galed,G. and Heres,A.(2009), Functional characterization of chitin and chitosan,Current Chemical Biology,3,203-230.

Awadalla, O.A. and Mahmoud, Y.A.G. (2005), New chitosan derivatives induced resistance to *Fusarium* wilt disease

through phytoalexin (Gossypol) production, Sains malaysiana, 34, 141-146.

Devi, K.M., Annapoorani, S and Murugesan, S. (2008), Antifungal activity of *Calotropis procera*, Madras Agric. J., 95, 7-12.

Du,Y., Zhao,Y and Yang,B.(2009), Preparation of water-soluble chitosan from shrimp shell and its antibacterial activity, Innovative Food Science and Emerging Technologies, 10,103-107.

Gaur,K., Rans,A.C., Chauhan,L.S., Sharma,C.S., Nema,R.K., Kori,M.L and Yashwant.S (2009), Investigation of Immunomodulatory potential of *Euphorbia neriifolia Linn* against Betamethasone induced Immunosuppression, IJPPR, 1, 8-11.

Goy,C.R., Britto,D., and Assis,O.B.(2009), A Review of the antimicrobial activity of chitosan, Polimeros:Cienciae Tecnologia., 19, 241-247.

Hong, K.NO., Samuel, P.M and Lee, K.S. (1989), Isolation and characterization of chitin from craw fish shell waste, J. Agri. Food chem., 37,575-579.

Lukovic,J., Malencic,D., Zoric,L., Kiprovski,B., Merkulov ,L.and Boza,P. (2009), Anatomical characteristics and antioxidant properties of *Euphorbia nicaeensis ssp.glareosa* , Cent.Eur.J.Biol, 4,214-223.

Marques, A., Encarnacao, S., Pedro, S. and Munes, M.L, (2008), *In vitro* antimicrobial activity of garlic,oregano and chitosan aginst *Salmonella enterica* , World J Microbial Biotechnol, 24,2357-2360.

NCCLS (National Committee for Clinical Laboratory Standards).(1997), Performance standards of antimicrobial susceptibility test, 6th edition , Approved Standard M2-A5, Wayne, PA, USA.

Parekh,J. and Chanda,S.(2008), *In vitro* antifungal activity of methanol extracts of some Indian medicinal plants against pathogenic yeast and moulds, African J. Biotechnology, 7, 4349-4353.

Rathore, P., Hejde, A., Ginjupalli, K and Upadhya, P.N. (2009), Evaluation of antifungal activity of additives to resilient liners: an *in vitro* pilot study, Trends Biomater, Artif.Organs, 23, 6-9.

Schlumbaum,A., Mauch,F., Vogeli,V. and Boller,J.(1986), Plant chitinase are potent inhibitors of fungal growth Nature,324, 365-367.

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