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Original Article

ANTIMICROBIAL ACTIVITY OF BINARY AND TERNARY COMPOSITES OF CHITOSAN AMENDED WITH NYLON 6 AND MONTMORILLONITE CLAY

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ABSTRACT

Objective: To evaluate the antimicrobial activity of binary and ternary composition of Chitosan along with Montmorillonite clay and Nylon 6 in various ratios and to identify a potential agent to use in an antimicrobial topical cream.

Methods: The antimicrobial properties using the following bacterial strains, i.e. *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* and the fungal strains, i.e. *Aspergillus niger, Candida albicans* and *C. tropicalis* were studied. Chloramphenicol and Ketoconazole were used as a control for bacteria and fungi respectively. The study was conducted using well diffusion method.

Results: Among the composites, Chitosan/Montmorillonite clay/Nylon 6 (2:1:1) has recorded the maximum zone of inhibition for *Escherichia coli, Pseudomonas* sp. *Aspergillus niger, Candida albicans* and *C. tropicalis* when compared to control. For *Staphylococcus aureus,* none of the compounds have provided any significant zone of inhibition.

Conclusion: The composite, Chitosan/Montmorillonite clay/Nylon 6 (2:1:1) can successfully used as a pharmaceutical agent in topical creams.

Keywords: Chitosan, Nylon 6, Montmorillonite Clay, Antibacterial property, Antifungal property.

INTRODUCTION

The studies of biopolymer films have received much attention because they are environmental friendly, alternatives to synthetic and non-biodegradable films. Chitosan is the deacylated form of chitin composed of glucosamine, which is one of the most abundant polysaccharides in nature [1, 2]. Because of its favorable properties such as enzymatic biodegradability, non toxicity, and biocompatibility, a variety of applications have been found either alone or blended with other polymers in the pharmaceutical industries [3-5]. Chitosan and its derivatives have been proposed as matrices in pharmaceutical formulations in the form of films, emulsions, troche, transmucosal devices and in drug delivery system [6-11]. At present, it has been commonly recognized that the biological activity of chitosan depends on its molecular weight, deacetylation degree, chitosan derivatization, degree of substitution, length and position of a substituent in glucosamine units of chitosan, pH of chitosan solution and the target organism [12].

Chitosan film containing bio-functional materials have been used as edible coatings for prolonged shelf life and preserve the quality of fresh foods [1, 3 and 13]. Due to its soluble nature in acidic solution, films can be readily prepared by casting or dipping, resulting in dense and porous structure [8]. Chitosan films have been tested as curative wound dressing and as scaffolds for tissue and bone engineering [7]. Recently many of the studies were reported on chitosan for its strong antimicrobial activity and antifungal activities [14-20]. The aim of the present work is to evaluate the antimicrobial activities of chitosan blended with clay and nylon 6 in different ratios.

MATERIALS AND METHODS

Preparation of chitosan

Chitosan (90%) procured from DNP International, Cochin was used to make all chitosan solutions. Chitosan solutions from 2 to 20 g/L were used for the experiments. The powdered chitosan was weighed and dissolved in 0.05 M or 0.01 M acetic acid

Preparation of Nylon 6

Nylon 6 in pellet form was obtained from DuPont and had a molecular weight of 19,000. One gram of nylon 6 dissolved in 88 % formic acid is mixed with 25ml of deionized water as working standard.

Preparation of clay

The suspension of montmorillonite (MM) clay was prepared by mixing 1 g of clay in 25 ml of deionized water and stirring in moderate speed for 20 min.

Composition of Binary and Ternary composition of Chitosan

The polymer blends of different composition were prepared using different ratios are as follows: chitosan/nylon 6 (1:1); chitosan/nylon 6 (1:2); chitosan/nylon 6 (2:1); chitosan/clay (1:1); chitosan/clay (1:2) and chitosan/clay (2:1). The ternary composition of the blends like, chitosan/nylon 6/clay (1:1:1); chitosan/nylon 6/clay (1:2:1) and chitosan/nylon 6/clay (2:1:1) were prepared.

Antibacterial and Antifungal activity

The assay is carried out by well diffusion method. Mueller Hinton Agar and Sabouraud's Dextrose Agar were used to study antibacterial and antifungal activity [21]. The respective plates were spread with specific cultures using a swab and wells were bored in each plate at the diameter of 6 mm each. The wells were filled with 25 µg of the composites/ well. The plates with bacterial cultures were incubated at 37°C for 24 h and the plates with fungal cultures were incubated at 28° C for 72 h for evaluation. The development of the zone of inhibition was compared with negative control and the differences were recorded [22]. Chloramphenicol (30µg/disc) and Ketoconazole were used as positive control for bacterial and fungal species. The assays were performed in triplicate.

RESULTS AND DISCUSSION

Antimicrobial Activities

Among the binary composites, the results showed that Chitosan and Montmorillonite clay (2:1) as possessing better antimicrobial property towards all the microbes studied except that of *Staphylococcus* sp. when compared with other binary composites. This was followed by Chitosan and Montmorillonite clay (1:1) against *Escherichia coli, Pseudomonas* sp. and *Aspergillus niger.* Chitosan and Nylon 6 (2:1) showed better activity for the species of *Candida* following Chitosan and Montmorillonite clay (2:1) among the binary composites.

Among ternary composites studied, Chitosan, Montmorillonite clay and Nylon 6 (2:1:1) showed the best activities with all three types of composites studied. The composite has also superseded that of the control except for *Staphylococcus* sp. The details on the zone of inhibition recorded for both binary and ternary composites of Chitoson, Montmorillonite Clay and Nylon 6 at different ratio is presented in Table 1.

Table 1: Antibacterial activity of Chitosan, Montmorillonite clay and Nylon 6

Microbial Species	*E. coli*	*Pseudomonas* sp.	*Staphylococcus* sp.	*A. niger*	*C. albicans*	*C. tropicalis*
Chloramphenicol (Positive Control - Bacteria)	38 ± 0.25	26 ± 0.05	37 ± 0.21	-	-	-
Ketoconazole (Positive Control - Fungi)	-	-	-	28 ± 0.12	26 ± 0.11	34 ± 0.1
0.1% DMSO (Negative control)	-	-	-	-	-	-
Binary Blends						
Chitosan and Nylon 6 (1:1)	29 ± 0.05	20 ± 0.11	25 ± 0.26	28 ± 0.15	26 ± 0.21	28 ± 0.21
Chitosan and Nylon 6 (2:1)	19 ± 0.21	16 ± 0.24	21 ± 0.05	36 ± 0.11	33 ± 0.12	36 ± 0.15
Chitosan and Nylon 6 (1:2)	5 ± 0.12	5 ± 0.12	5 ± 0.12	27 ± 0.11	24 ± 0.11	23 ± 0.12
Chitosan and Montmorillonite clay (1:1)	38 ± 0.05	31 ± 0.11	32 ± 0.26	37 ± 0.11	32 ± 0.12	35 ± 0.11
Chitosan and Montmorillonite clay (1:2)	25 ± 0.12	23 ± 0.21	33 ± 0.32	29 ± 0.22	24 ± 0.11	22 ± 0.11
Chitosan and Montmorillonite clay (2:1)	39 ± 0.21	36 ± 0.24	32 ± 0.05	38 ± 1.12	36 ± 0.12	37 ± 0.11
Tertiary Blends						
Chitosan, Montmorillonite Clay and Nylon 6 (1:1:1)	39± 1.05	32± 0.02	34± 0.22	38 ± 1.12	34 ± 0.31	36 ± 0.31
Chitosan, Montmorillonite clay and Nylon 6 (1:2:1)	27± 1.22	26± 0.02	35± 0.31	31 ± 1.02	27 ± 0.22	25 ± 0.33
Chitosan, Montmorillonite clay and Nylon 6 (2:1:1)	39.5±1.25	38.2± 0.01	33.2± 1.25	38 ± 0.12	37 ± 0.22	38 ± 0.22

The blend with low content of chitosan showed lesser antibacterial effect for all the bacteria studied. At the same time, the antimicrobial activity of the blended composites increases with increasing content of chitosan in polymer blends. This phenomenon may be related to the presence of more amount of net positively charge in the chitosan macromolecules. But this is less pronounced in case of blend with synthetic polymer viz., nylon 6 where it is less porous. The same is more pronounced in case of blend with natural polymer, viz., clay where it is more porous when compared to nylon 6. From the result it was observed that all the polymer blends showed significant activity against the bacterial strains studied, except chitosan/nylon 6 (1:2).

The results suggested two different mechanisms of chitosan and target microorganism interaction, the first: adsorption of chitosan to cell walls, membrane disruption and cell leakage which is mainly connected with high molecular weight chitosan, the second: penetration of chitosan into living cells leading to inhibition of various enzymes and interference with synthesis of mRNA and proteins [12]. However, the actual mechanism of inhibition activity of chitosan and its derivatives is not fully understood yet.

The most feasible hypothesis is a change in cell permeability due to interaction between the positively charged chitosan and negatively charged microbial cell membranes, which prevents the transport of essential solutes into the cell and results in leakage of proteinaceous and other intracellular components, thus killing the bacterial cell [4, 17]. This hypothesis has been well established when we change the polymer nylon 6 to clay where there is more possibility for permeable positive charges and the interaction is well established in more porous nature of the polymer compared to synthetic polymer.

With increasing content of chitosan this inhibition effect is low whereas with increasing content of clay the inhibition effect is high and this goes well with our hypothesis that more porous nature will help in the migration of positive charges within the matrix and kills the bacteria. The bacterial growth inhibition is attributed to the diffusion of entrapped chitosan from the polymer blend to the culture medium where this will be more plausible in case of natural polymer blend of chitosan and clay unlike the polymer blend of chitosan with synthetic polymer nylon 6 where the diffusion of entrapped chitosan is less to the culture medium.

CONCLUSION

The present study was conducted to find out the potency of binary and ternary composites of Chitosan, Montmorillonite clay and Nylon 6 at different composition. The study concludes that the composite, Chitosan/Montmorillonite clay/Nylon 6 (2:1:1) possess better antimicrobial activity when compared with standards. Thus, the composite can be successfully employed in the pharmaceuticals like band aids and antimicrobial topical creams.

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