Antimicrobial properties of biodegradable polymer based ternary blend for wastewater treatment

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Abstract

Biodegradable based ternary blends has good antimicrobial properties and it has been verified against gram / negative *E. coli*, *Pseudomonas sp. aeruoginosa,* and gram-positive S*taphylococcus sp*. S. *aureus bacteria.* **T**he comparison of binary and ternary blended reveals that more inhibition zone efficiency is found for ternary blended. Chitosan/nylon 6/clay (1:1:2), (2:1:1) and (1:2:1) respectively were reported. The binary blends inhibition zone chitosan/clay (2:1) recorded the maximum zone of inhibition in comparison with the standard *chloramphenicol* against *E. coli* (39 mm), *Pseudomonas sp. aeuroginosa* (36 mm), followed by *S.aureus* (22 mm). Most of the polymer blends showed higher inhibitory activity against Gram-negative strains than the Gram-positive bacteria. The antifungal activity of the chitosan polymer blends is seen and this activity is more pronounced against fungi. The inhibition was stronger in the order of chitosan/nylon 6/clay (2:1:1) followed by Chitosan/nylon 6/clay (1:1:1) and (1:1:2) against *Aspergillus niger, Candida albicans and candida tropicalis.* A higher inhibitory zone was recorded against *Aspergillus niger* (36 mm), *Candida albicans* (33 mm), *and Candida tropicalis* (36 mm) by chitosan/clay (2:1:1) when compared with standard *amphoterecin B*. Meanwhile Chitosan/nylon 6/clay (1:2:1) showed less activity. The present study is aimed to fetch the knowledge about the antimicrobial properties of biodegradable polymer based ternary blends in detail.

Keywords: Chitosan; Nylon 6; Clay; Antimicrobial activity; Antifungal activity.

1. Introduction

The attempts of biodegradable polymer films have received much attention for the environmentally friendly; alternatively to substitutes to synthetic and non-biodegradable films.

Chitosan and chitin are deacylated and composed of glucosamine, which is the most abundant polysaccharide in the environment. The subsequently of its extensive properties such as enzymatic biodegradability, non-noxiousness, and biocompatibility, different applications have been initiated blended, or not with other polymers in the chemical industries [1-3]. Chitosan relatives derivatives have been proposed as matrices in formulations in the form of films, emulsions other devices, and drug delivery methods [4]. To recognize that the biological activity of chitosan depends on its molecular weight, pH of chitosan and the target organism, chitosan derivatization, length, and position of a substituent in glucosamine units [5]. Fresh food quality of coating for shelf life prolonged, and it is used in the chitosan film containing bio-functional materials [6]. The solution is soluble in acidic conditions, films can be prepared by casting or dipping, and the way of a dense and porous structure. Tissue and bone engineering have been scaffolds for wound dressing and chitosan films. Nowadays many research studies were reported on chitosan related to strong antimicrobial activity and antifungal activities [7-10]. Prakash and Arungalai Vendan synthesized the ternary blends that consists of chitosan, nylon 6 and montmorillonite using solution blending method with the aid of glutaraldehyde. They also reported that their synthesized ternary blends was able to remove the trace metals from industrial waste water [11]. The present work aims to evaluate the antimicrobial activities of chitosan blended with clay and nylon 6 in different ratios.

2. Materials and Methods

2.1 Preparation of chitosan

Pure chitosan getting from Indian kinds of seafood and DNP International, (city and state) Cochin which is 92% deacetylate, to make the chitosan sample and kept in stock solution as it's used. From the solutions to prepared (2 to 20 g/L) and diluted with other polymer solutions like nylon 6 and clay and used in experiments. To weigh and dissolved in 0.05 or 0.01 M acetic acid, depending on the pH required.

2.2 Preparation of nylon 6 and Clay

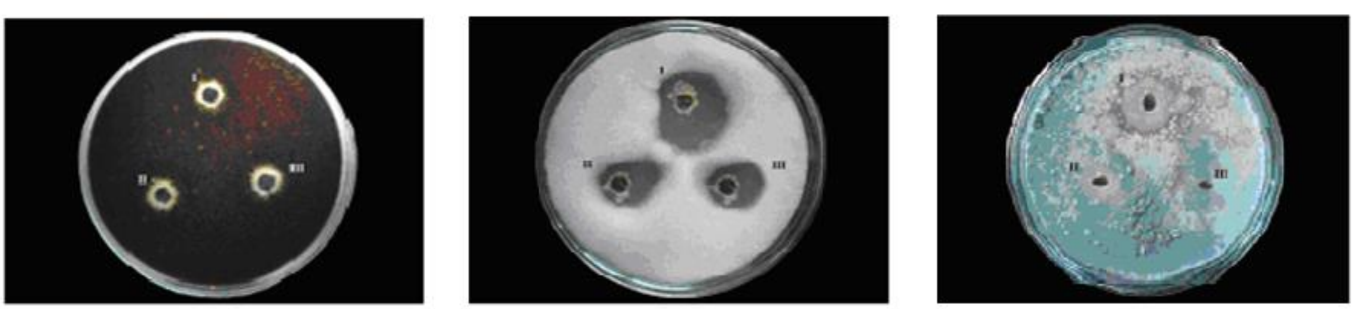
Nylon 6 sample in pellet form received from DuPont and had higher molecular weight. All other materials from Analytical Reagent, Merck (formaldehyde and glutaraldehyde were used as received. Clay received from Aldrich (<150 μm) was used.

3. Antimicrobial Activity

Ternary blends have been taken by *vol./vol*. However, in this method agar plates are inoculated with test microorganisms from the standardized inoculums. Antimicrobial analysis using paper filter discs (about 6 mm in diameters) to prepare desired concentrations are placed on the agar surface. To suitable conditions to keep the Petri dishes incubated. Generally, the antimicrobial test diffuses into the agar and inhibits germination and growth of the test microns and the diameters of inhibiting growth media, by using Clinical and Laboratory Standards Institute standards to form the size of the period of incubation with temperature. Ternary blends have been taken by *vol./ vol*. the pits are filled with biodegradable polymer blends, shown in Fig. 1 from the results of the ternary blending ratio as ChitosanS/Nylon 6 (1:1), (1:2), and (2:1); and Ffig.2 from the results of binary blending ratio as Chitosan/ Clay (1:1), (1:2) and (2:1) at the concentration of (25 µg/well respectively). They incubated at 37 ^oC for 24 hours at the bacterial plates. The plates holding fungal cultures were incubated at 28 ^oC for 72 hours. The zone of inhibition was measured by subtracting the well diameter from the total inhibition zone [12-14]. *Chloramphenicol* (30 µg/disc) and *Amphotericin B* (100 units/well) were used as a positive control for bacterial and fungal species, the assays were performed in triplicate.

a. Escherichia coli *b. Pseudomonas aeruoginosa* *c. Staphylococcus aureus*

Fig. 1(a, b, c): Antifungal assay of chitosan and nylon 6 with glutaraldeyde (Agar well diffusion method)



a. Aspergillus niger *b. Candida albicans* *c. Candida tropicalis*

Fig. 2(a, b, c): Antifungal assay of chitosan and montmorillonite clay with glutaraldeyde (Agar well diffusion method)

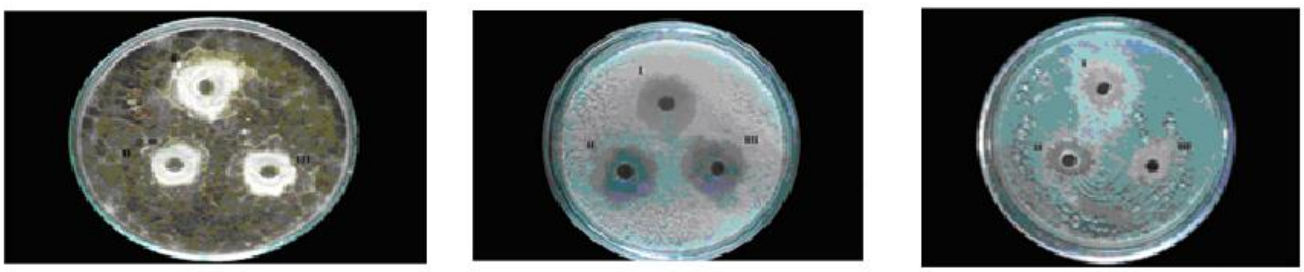
4. Results and Discussion

The tests to evaluate the antifungal properties generally fall into two categories: agar diffusion test (qualitative method) and dynamic shake test (quantitative method). In the present study, the first method was followed which has been already described and the results are presented in Table 1.

Table 1. Antifungal activity of chitosan, montmorillonite clay, and nylon 6 with glutaraldeyde blends

Binary blends	***Aspergillus niger***	***Candida albicans***	***Candida tropicalis***
Control (Amphoterecin B)	28 + 0.12	26 + 0.11	34 + 0.1
0.1% DMSO	-	-	-
Chitosan and nylon 6 (1:1+GA)	36 + 0.15	31 + 0.21	36 + 0.21
Chitosan and nylon 6 (1:2+GA)	27 + 0.11	24 + 0.11	23 + 0.12
Chitosan and nylon 6 (2:1+GA)	36 + 0.11	33 + 0.12	36 + 0.15
Chitosan and montmorillonite clay (1:1+GA)	37 + 0.11	32 + 0.12	35 + 0.11
Chitosan and montmorillonite clay (1:2+GA)	29 + 0.22	24 + 0.11	22 + 0.11
Chitosan and montmorillonite clay (2:1+GA)	38 + 1.11	36 + 0.12	37 + 0.11
Chitosan, montmorillonite clay and nylon 6 (1:1:1+GA)	38 + 1.12	34 + 0.31	36 + 0.31
Chitosan, montmorillonite clay and nylon 6 (1:2:1+GA)	31 + 1.02	27 + 0.22	25 + 0.33
Chitosan, montmorillonite clay and nylon 6 (2:1:1+GA)	38 + 0.12	37 + 0.22	38 + 0.22

From the results of the investigations it is seen from the Fig..3 that in the case of anti fungi activity, the inhibition was stronger in the order, of CS, clay (2:1+GA), CS, clay, and NY 6 (1:1:1+GA), (2:1:1+GA), followed by CS, clay (1:1+GA), CS, NY 6 (1:1+GA) and CS, clay (1:2+GA) against *Aspergillus niger*, *Candida albicans and Candida tropicalis.* A higher inhibitory zone was recorded against Aspergillus niger (36 mm), *Candida albicans* (33 mm), and *Candida tropicalis* (36 mm) by chitosan and montmorillonite clay (2:1+GA) when compared with standard amphotericin B. Meanwhile, chitosan and nylon 6 (1:2+GA) showed lesser activity [15].



a. Aspergillus niger *b. Candida albicans* *c. Candida tropicalis*

Fig. 3 (a, b, c): Antifungal assay of chitosan, montmorillonite clay and nylon 6 with glutaraldeyde (Agar well diffusion method)

The antifungal activity of the ternary blend is higher than the binary blend. The antifungal property of the synthesized chitosan polymer blends may be due to the damage made to the plasma membrane [16]. The prepared chitosan polymer blends showed higher antimicrobial activity against the selected fungi than the bacterial species. This is in agreement with the previous studies reported in the literature. Park [17] explored the antimicrobial activity of the adapted CS against some fungi, *C.albicans*, *A.flavus,* and *F.oxysporum*, and bacteria *B.subtilis*, *E.coli*, *and S.aureus*, it was found that the modified products were generally active toward fungal species more than the bacterial species. The modified chitosan derivatives have a greater effect on the fungi than the bacteria based on the larger clear inhibition zones Park.

The influence of chitosan is understood from the inhibition zone diameter data. It becomes larger with more chitosan content in the blend with nylon 6 and montmorillonite clay. Nylon 6 membrane alone cannot exhibit antibacterial activity. But if the nylon 6 content of the membrane is increased in the blend the flat dense surface of the chitosan membrane became slowly undulating and this helps in promoting an atmosphere conducive to bacterial adhesion and colonization[18]. By the incorporation of nylon 6 in the chitosan membrane, the surface becomes rougher and this is the reason for more conduciveness's the growth of cellular and bacterial colonization. In the case of montmorillonite clay blended with chitosan, the antibacterial property is more than chitosan blended with nylon 6 on two counts. (i) The montmorillonite clay helps in creating more surface for the growth of bacteria which will be attacked by chitosan and (ii) Cationic surfactants used for montmorillonite clay modified chitosan promotes the biocidal activity[19-20]. The biocides are exchangeable cations in the montmorillonite clay, chitosan blend and these can migrate from the montmorillonite clay and thereby affect bacteria as soluble antimicrobial agents. This biocidal migration is only partially responsible for the antimicrobial activity of the montmorillonite clay, nylon 6 blended with chitosan.

5. Conclusions

The ratio of a binary blended system has strong antibacterial activity stability, but it is not long-lasting. In contrast, a ternary blended system has a long history of antimicrobial activity stability. The antimicrobial activity of ternary blended compositions increases with the increasing content of chitosan in polymer blends. This is less pronounced in the biodegradable polymer blended with synthetic polymer viz., nylon 6 where there is a less porous nature. Antimicrobial activity is more pronounced in the chitosan blended with natural polymer viz., clay where there is a more porous nature compared to nylon 6. Total polymer blends show significant activity against the tested bacterial strains except CS/NY 6/clay is (1:2:1) and CS/clay/NY 6 is (2:1:1) and recorded the maximum zone of inhibition in comparison with the standard Chlorophenicol against *E. Coli*, *Psedomonas sp.* and *Aeuroginosa*. It is observed from the antimicrobial studies that most of the polymer blends showed higher inhibitory activity against gram-negative strains than the gram-positive bacteria.

Conflict of interest:

The authors report there are no conflicts of interest.

Data availability statement: The authors confirm that the data supporting the findings of this study are available within the article.

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