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Assessment of N,N-dimethylamine-N-ethylamine chitosan for antimicrobial activity

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ABSTRACT

Antibacterial and antifungal activity of synthesized N-N-dimetylamine N-ethylamine chitosan were studied against E.coli, P.aeruginosa, S. aureus, S. pyogenes and A.niger, A. clavatus and C. albicans respectively. The result showed that the remarkable inhibition of the bacterial growth against the tested organisms. The microbial activity of N-N-dimetylamine N-ethylamine chitosan was due to presence of amino groups along the polymer chain and hence can be used in the development of new pharmaceutical activities.

Key words: N-N-dimetylamine N-ethylamine chitosan, in *vitro* antibacterial activity, antifungal activity.

INTRODUCTION

Chitosan is natural biodegradable biopolymer, used to produce value added products because it is rich in protein, carotenoides and chitin[1][2]. It has antimicrobial property effective against a wide variety of pathogenic microorganisms, however its activity depends on presence of active amino groups [3][4]. It is assumed that antimicrobial action is due to positively charged amino groups which interacts with negatively charged bacterial cell membrane and chitosan with chelating effect which inhibits the growth of microorganism[5][6].

The aim of this work was to characterize the antimicrobial activity of newly synthesized material N-N-dimethylamine N-ethylamine chitosan[7].

MATERIALS AND METHODS

700 mg of chitosan dissolved in 1% w/v acetic acid (original pH 3.7±0.5). Then specific amounts of acetaldehyde added to the chitosan solution and after 1.5 hours of stirring, the pH of solution was adjusted to 4.5 by adding ethylamine solution. Then 2.0ml of a 10% sodium borohydride solution were added and magnetically stirred for 2hours. The first step was to obtain Mono-ethylamine chitosan precipitate. The precipitate was washed with distilled water.

200mg of ethylamine chitosan were dispersed in 10ml of NMP for 5hours.Then methylamine (2ml) methyl iodide (5ml) and sodium iodide (600mg) were added to the dispersion. The reaction was carried out with stirring for 5 hours at 60ºC. Finally, acetone was added to precipitate the quaternized chitosan derivative[8]. All the other materials was purchased from Sigma-Aldrich and used without further purification.

Test Microorganisms and Growth Media

The bacterial strains *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes* and *fungal strains Aspergillus niger, Aspergillus clavatus* and *Candia albicans* were chosen based on their pharmacological importance[9].

The bacterial cultures on nutrient agar and fungal stock cultures on potato dextrose agar medium were incubated for 24 hours at 36^{o}C, stored at 4^{o}C. The bacterial strains were grown in agar plates at 36^{o}C whereas the yeast and moulds were grown in Sabouraud dextrose agar and potato dextrose media, respectively at 29^{o}C. The stock cultures were maintained at 4^{o}C.

Antimicrobial Activity

Determination of Zone of inhibition method

Antimicrobial and antifungal activities against pathogenic bacteria and fungi were examined by standard disc diffusion method in *vitro* for aqueous solution (double distilled water used). Antimicrobial activity testing was carried out by using agar cup method.

The set of five dilutions (5, 25, 50, 100, 250µg/ml) of N-N- dimethylamine N-ethylamine chitosan were prepared in double distilled water and controlled experiments were carried out under similar condition. The zones of growth inhibition around the disks were measured after 24 to 30 hours of incubation at 36^{o}C for bacteria and 48 to 96 hours for of incubation for fungi at 30^{o}C. The sensitivities were determined by measuring the size of inhibitory zones on agar surface around the discs.

RESULTS

Antibacterial and antifungal potential of N-N-dimethylamine N-ethylamine chitosan increased linearly with increase in concentration (µg/ml). The growth inhibition zone measured ranged from 21 to 30 mm for all sensitive bacteria and ranged from 24 to 30 mm for fungal strain. The result (Table1. & Figure 1,2,3, 4.) revealed that for bacterial activity *E.coli* and *S.pyogenes* are more sensitive as compared with *S. aureus* and *P.aeruginosa* and for fungal activity(Table 2. & Figure 5,6,7) *A. niger* shows good result as compare with *C. albicans* and *A.clavatus*.

Table1. Antibacterial test against bacterial test organisms

Microrganism	Zone inhibition in mm				
	N-N-dimethylamine N- ethylamine chitosan concentration				
	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml
E.coli	-	22	23	24	28
S. pyogenes	-	23	24	27	28
S. aureus	-	25	26	27	30
P. aeruginosa	-	23	25	28	30

Table 2. Antibacterial test against fungal test organisms. (- indicates no zone of inhibition).

Microrganism	Zone inhibition in mm				
	N-N-dimethylamine N-ethylamine chitosan concentration				
	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml
A. niger	-	27	28	29	30
C. albicans	-	25	26	28	30
A. clavatus.	-	26	27	29	30

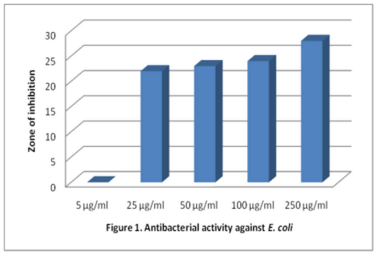

Figure 1. Antibacterial activity against *E. coli*

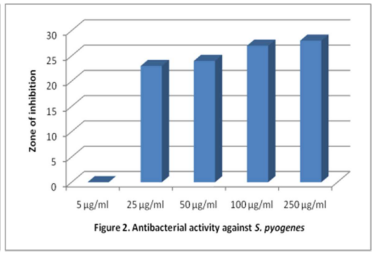

Figure 2. Antibacterial activity against *S. pyogenes*

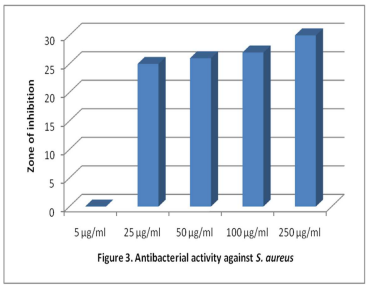

Figure 3. Antibacterial activity against *S. aureus*

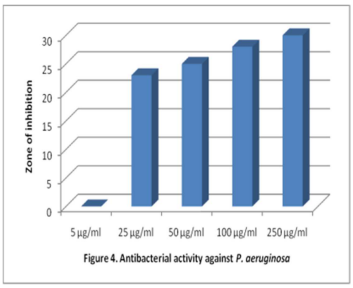

Figure 4. Antibacterial activity against *P. aeruginosa*

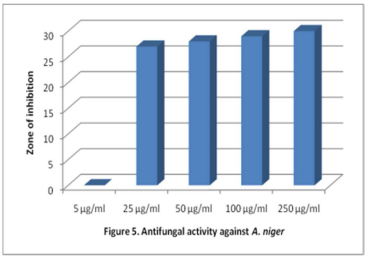

Figure 5. Antifungal activity against *A. niger*

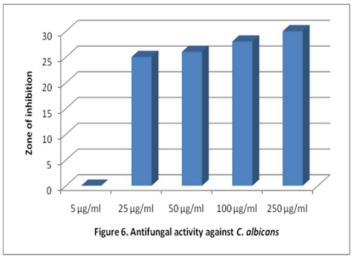

Figure 6. Antifungal activity against *C. albicans*

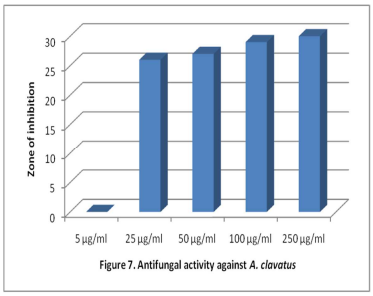

Figure 7. Antifungal activity against *A. clavatus*

CONCLUSION

The N-N-dimethylamine N-ethylamine chitosan was found to be active on most of the clinically isolated microorganisms and fungi.

REFERENCES

[1] P. Lertsutthiwong, N.C. How, S. Chandrkrachang, W.F. Stevens. *J. Met. Mater. Miner.*, **2002,**12(1),11-12.
[2] B. Li, X. Wang, R. Chen, W. Huangfu, G.Xie. *Carbohydrate polymers.*, **2008.,** 72, 287-292.
[3] P. K. Dutta, S. Tripathi, G. K. Mehrotra, J.Dutta. *Food Chem.,* **2009,**114,1173-1182.
[4] M. Aider. *LWT Food science and technology*, **2010**, 43, 837-842.
[5] T. Hauge, H.Chen, W. Ouyang, C. Martoni, B. Lawuyi, A.M.Urbanska, S.Prakash. *Mol. Pharm.*, **2005**, 2(1), 29-36.
[6] Y. Pranoto, S. K. Rakkshit, V. M. Salokhe. *LWT Food science and technology.*, **2005**, 38,859-865.
[7] P.R. Bhagat, S.A. Aswar, V.S.Yeul, *Res.J.Chem.Environ*, **2015**,19(4), 59-62.
[8] S.A Aswar, P.R Bhagat. *Int. J. of Knowledge Engineering*, **2012,** (3)1, 135-136.
[9] W.A. McCracken, R.A. Cowsan. *Clinical and oral microbiology. New York: Hemisphere publishing corporation*; **1983**. 512.