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ANTIMICROBIAL POTENTIAL OF CHITOSAN AND *MORINGA OLEIFERA* LEAF POWDER

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ABSTRACT

Chitosan is a useful and interesting bioactive polymer, with reactive amino groups, which offer possibilities of chemical modifications and the formation of a large variety of useful derivatives. The method reported by Maulin was used and as well modified for synthesizing chitosan. Crayfish sample was macerated, demineralized, deproteinized and washed with distilled water to neutral pH. Chitin was immersed in 12.5M NaOH and washed to neutral pH. The synthesized chitosan was characterized using Fourier transform infrared (FTIR) spectroscopy. The results indicated a more deacetylation with the presence of amine and hydroxyl functional groups. The chitosan was soluble in dimethylsulphuroxide (DMSO) and 1% acetic acid. DMSO has no activity on the tested organisms but 1% acetic acid has antimicrobial activities on the tested organisms as shown in this result. Moringa leaf was washed, macerated and grind to powder and then tested against the test isolates. The results showed that chitosan and Moringa leaf powder have high antimicrobial efficiency on the tested isolates having expected physical and chemical properties suitable for food and pharmaceutical applications.

Key word: Antimicrobial, chitosan, *moringa oleifera*, synthesis

INTRODUCTION

The name `chitin' is derived from Greek word `chiton', meaning a coat of mail [1]. It is a β-1, 4-D-glucosamine polymer derived from alkaline chitin deacetylation. Chitin is a structural component of fungi, insects, and crustaceans insoluble in many solvents, but soluble in organic acids [2].

The use of natural antimicrobials in place of conventional ones due to their effects on health has got serious attentions. Chitosan is a natural biodegradable, and non-toxic biopolysaccharide derived from chitin which has the potential to be used as a natural antimicrobial agent. Chitosan exhibits high

antimicrobial activity against wide variety of pathogenic and spoilage microbes [3]. Antimicrobial activity of chitosan is influenced by the type of chitosan, the degree of polymerization, the type microbes, the environmental conditions, and presence of the other components. The use of chitosan in food systems should be on the knowledge of the mechanisms of its antimicrobial mode of action [4].

The mechanism of chitosan antimicrobial activity is that, it interacts with negatively charged phospholipid components of fungi/bacteria membrane, acts as a chelating agent and penetrates the cell wall of fungi/bacteria and bind to the DNA [2, 5]. The electrostatic interaction between positively charged sites of chitosan and the negatively charged microbial cell membranes assumed to be responsible for cellular lysis and assumed to be the main antimicrobial mechanism [2, 6]. Charged chitosan can also interact with essential minerals, hence, interfere on the microbial growth [7]. It is expected that polymers with higher charge densities resulted in improved antimicrobial activity. Antimicrobial effectiveness of chitosan is strongly dependent on concentration [8].

Chitosan antimicrobial potential is influenced by its molecular weight, degree of substitution, concentration, microbe types, and types of functional groups in chitosan derivatives chains [9]. The antimicrobial activity is contributed by the polycationic nature of chitosan. Chitosan exhibits natural antimicrobial potential without chemical modification [10].

The primary amine groups in repeating units of chitosan gave it several properties like antimicrobial activity, antitumor activity; Chitosan nanoparticles (ChNP) compounds exhibit high antimicrobial activity against all microorganisms compared to chitosan and chitin [11].

Antibiotic-resistant strains is challenging poultry industry to find alternative of control and consequently, continuous studies on methods to control food-borne pathogens [12 &13].

Chitosan has antimicrobial activity against many bacteria [2, 11, 14-16], like, *in vitro* and *in vivo* (mice) that chitosan oligosaccharides have an antimicrobial effect on the Gram-negative bacterium *Vibrio vulnificus*, which causes sepsis and gastrointestinal illness in humans [17]. Most of the studies are related to the *in vitro* effect of chitosan in reducing bacteria, not considering its effects in the presence of organic matter and more importantly under *in vivo* conditions.

Chitosan is widely used as an antimicrobial agent either alone or blended with other natural polymers to broaden its antimicrobial applicability, to broaden its applicability, comprehensive knowledge of its potential is necessary [18].

Moringa oleifera is the mostly and widely cultivated species of the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan [19] which is widely used for

combating microbial infections; anti-inflammation, sexually-transmitted diseases, malnutrition, and diarrhea. *Moringa* have been recognized by folk medicine practitioners as having value in the treatment of tumors [20]. The aim of this research is to synthesize and study the antimicrobial potential of *Moringa* and chitosan from crayfish with the effect of solvent on their antimicrobial activities.

EXPERIMENTAL

The Maulin's method was adopted for the chitosan synthesis and heat was excluded with an increase in time in the Maulin's modified method [18]. *Moringa* leaf was collected, washed, dried and macerated to powder and its antimicrobial activity determined.

Schematic diagram of chitosan synthesis from crayfish

Characterization

Chitosan was characterized using FTIR spectroscopy

Antimicrobial Activity of Chitosan and Moringa Leaves Powder.

The tested isolates were obtained from the Microbiology Laboratory of NILEST, Zaria.

Antimicrobial Activity Determination

The method reported by Abdullahi, *et al.*, [21] was employed for the antimicrobial potentials.

RESULTS AND DISCUSSION

Chitosan samples were prepared with different reaction conditions chosen. The chitosan were both obtained as white to light red solid powder, insoluble in water but soluble in DMSO and acetic acid after demineralization deproteinization and deacetylation.

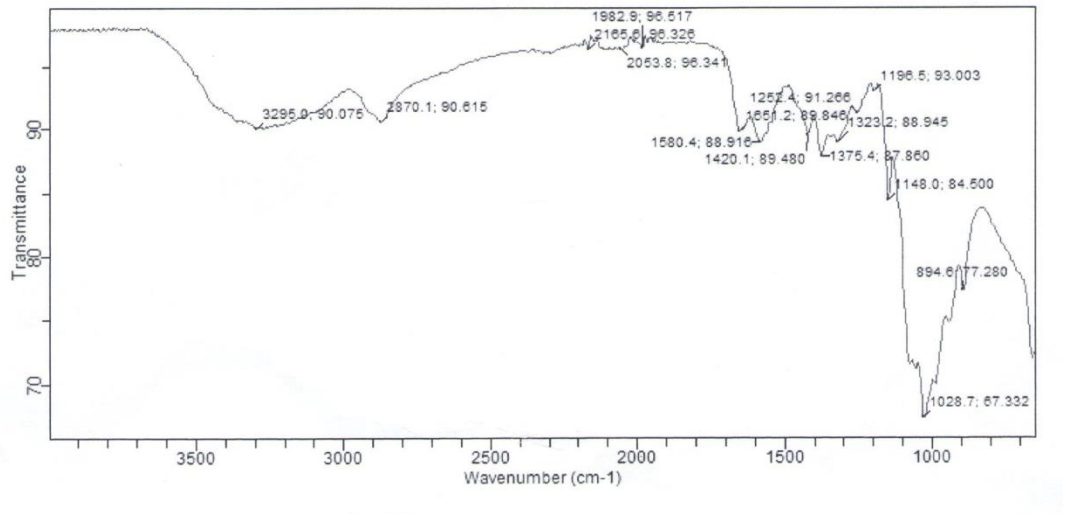

Figure 1: Chitosan FTIR for the adopted method

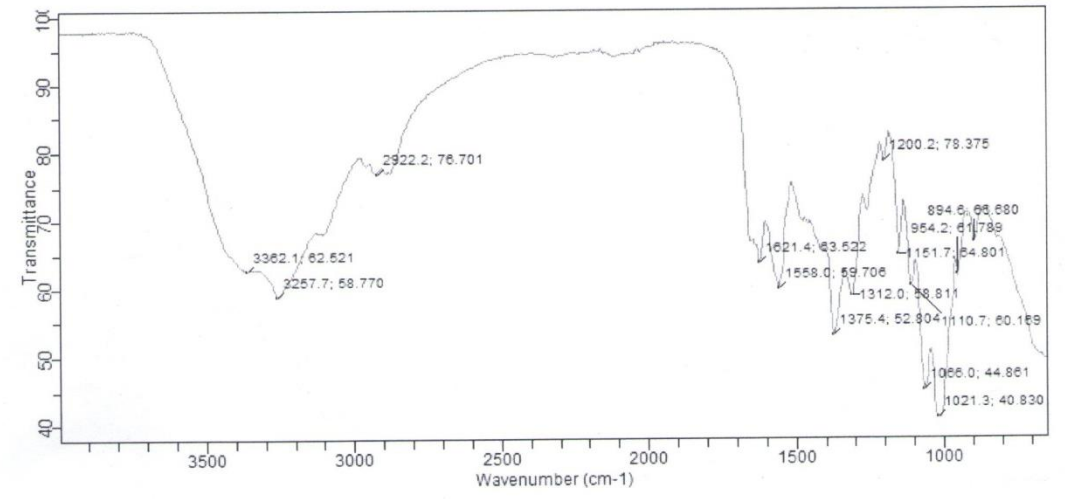

Figure: 2 Chitosan FTIR for Maulin's modified method

Characterization of Chitosan

FTIR study of chitosan was performed to characterize the chemical structure. In the adopted method (figure 1) spectrum, a band of 3295cm^{-1} was obtained which corresponds to the peak of the O-H stretch.

A 2870cm^{-1} peak of C-H stretch was obtained. A 1651 cm^{-1} peak of C-N stretch was obtained. A 1029 cm^{-1} peak of C-O-C stretch was obtained. In the Maulin's modified method (figure 2) spectrum, a band of 3362 cm^{-1} was obtained which corresponds to the peak of O-H stretch overlapped on N–H stretch (strong). A 2922 cm^{-1} peak of C-H group was obtained. A 1621 cm^{-1} peak of C-N stretch was obtained. A 1055 cm^{-1} peak of C-O-C stretch was obtained.

Table 1. The zones of inhibition of chitosan and *Moringa oleifera* leaf powder in mm

S/N	*microbes*	MD (200mg/mL)	MA (200mg/mL)	CD (200µg/mL)	CA (200µg/mL)	A	D
1	*S. aureus*	11	18	10	20	22	0
2	*S. typhi*	13	17	12	20	20	0
3	*P. bulgaris*	19	17	11	19	25	0
4	*E. coli*	15	19	14	21	24	0
5	*S. pnuemoniae*	21	18	12	19	20	0
6	*C. albicans*	12	27	14	28	30	0

Key: D=dimethylsulphuroxide (DMSO), A=1% acetic acid, MD = Moringa in DMSO, MA = Moringa in 1% acetic acid, CD = Chitosan in DMSO, CA= Chitosan in 1% acetic acid

Table 2.The minimum inhibition concentration (MIC)

Test microbes	MD (mg/ml)					MA (mg/ml)					C D (µg/ml)					CA (µg/ml)				
	200	100	50	25	12.5	200	100	50	25	12.5	200	100	50	25	12.5	200	100	50	25	12.5
S. aureus	+	++	+++	++++	+++++	--	--	--	--	--	+	++	+++	++++	+++++	--	--	--	--	--
S. typhi	--	+	++	+++	+++	--	--	--	--	--	+	++	+++	++++	+++++	--	--	--	--	--
P. bulgaris	--	--	-	+	++	--	--	--	--	--	+	++	+++	++++	+++++	--	--	--	--	--
E. coli	--	-	+	++	+++	-	-	-	-	-	-	+	++	+++	++++	-	-	-	-	-
S. pneumonia	-	-	-	-	-	-	-	-	-	-	+	++	+++	++++	+++++	-	-	-	-	-
C. albicans	+	++	+++	++++	+++++	-	-	-	-	-	-	+	++	+++	++++	-	-	-	-	-

Key: +=Growth level, - =No growth, MD = Moringa in DMSO, MA = Moringa in 1% acetic acid, CD = Chitosan in DMSO, CA= Chitosan in 1% acetic acid

Table 3.The minimum bactericidal/fungicidal concentration (MBC/MFC)

S/N	Test Organisms	MD (mg/ml)					MA (mg/ml)					C D (µg/ml)					CA (µg/ml)				
		200	100	50	25	12.5	200	100	50	25	12.5	200	100	50	25	12.5	200	100	50	25	12.5
1	S. aureus	+	++	+++	++++	+++++	-	-	-	-	+	+	++	+++	++++	+++++	-	-	-	-	+
2	S. typhi	+	++	+++	++++	+++++	-	-	-	-	+	+	++	+++	++++	+++++	-	-	-	-	+
3	P. bulgaris	-	-	+	++	+++	-	-	-	-	+	+	++	+++	++++	+++++	-	-	-	-	+
4	E. coli	-	+	++	+++	++++	-	-	-	-	+	+	++	+++	++++	+++++	-	-	-	-	+
5	S. pneumonia	-	-	-	-	+	-	-	-	-	+	+	++	+++	++++	+++++	-	-	-	-	+
6	C. albicans	+	++	+++	++++	+++++	-	-	-	-	+	+	++	+++	++++	+++++	-	-	-	-	+

Key: +=Growth level, - =No growth, MD = Moringa in DMSO, MA = Moringa in 1% acetic acid, CD = Chitosan in DMSO, CA= Chitosan in 1% acetic acid

The need for new and effective antimicrobial agents with broad-spectrum of activity from natural sources is on the increase [22]. Chitosan is produced by alkali deacetylation of chitin (CHI) as reported by Maulin. Chitin and chitosan attracts interest due to its biocompatibility, biodegradability, and non-toxicity [23]. Figure 1 shows the FTIR result of chitosan synthesized with method as reported by Maulin, and figure 2 shows the FTIR result of the modified method, both of which shows pronounced peaks of –OH stretch, -CH stretch, -C-N stretch, C-O-C stretch and –NH stretch. The nature of solvent used in dissolving any antimicrobial agent has effect on their antimicrobial potency as shown in Tables 1-3. 1% acetic acid has antimicrobial activity while dimethylsulphoxide (DMSO) has no antimicrobial activity respectively. The activity of the compound dissolved using DMSO is the actual antimicrobial activity while that dissolved using 1% acetic acid is the potency of the compound and that of the acid as shown in Tables 1-3 respectively. Chitosan and *Moringa* leaf powder showed high efficiency as antimicrobial agents against the test isolate used in this study. Chitosan activity is based on the concentration, degree of deacetylation, source and molecular weight. The reduction in the antimicrobial potentials of *Moringa* powder and chitosan dissolved in acetic acid could be as a result of the acetic acid denaturing the chitosan and Moringa powder used on the isolates as shown in Tables 2 and 3 respectively..

CONCLUSIONS

Chitosan is a useful and interesting bioactive polymer. It can readily be derivatized by utilizing the reactivity of the primary amino group and the primary and secondary hydroxyl groups to find applications in different areas like food, biomedical, water treatment with reduced toxicity and pharmaceuticals. A modified procedure for synthesizing chitosan was used which shows high efficiency in its antimicrobial property on the test isolates. *Moringa oleifera* is known for its therapeutic function. Moringa leaf powder and the synthesized chitosan were observed to be good antimicrobial agents from the analysis and can be used to discover antimicrobial agent for developing new pharmaceuticals to control human pathogenic bacteria responsible for severe illness in immune-compromised patients.

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