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Synergistic-Antagonistic Antibacterial Potential of Chitosan Composites with *Moringa oleifera* Leaf Powder

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ABSTRACT: Chitosan is very useful in everyday life in adsorption, cosmetics, pharmaceuticals, flocculants, anticancer and antimicrobial.In this study, chitosan was synthesized from chitin extracted from crayfish. The methods such as deproteinization, demineralization, and deacetylation respectively were used in the synthesis of chitosan from crayfish. Antimicrobial activity was studied and it was found that chitosan and *Moringa*leaf powder were good in inhibiting the growth of microorganisms; confirmed by the results obtained from the experiments. In evaluating the antimicrobial activity, the serial dilution method was used towards *Escherichia coli,* Staphylococcus*aureus, Salmonella typhi, Proteus bulgaris and Streptococcus* pneumonia. The antibacterial activity of chitosan composite with the leaf powder of *Moringa oleifera* Lam., was determined, using well diffusion, minimum inhibitory concentration (MIC) and minimum bactericidal concentration determination method. The composites show the synergistic effect at the higher chitosan to lower Moringa concentration and antagonistic effect at higher Moringa to lower chitosan concentrations in all the test organisms. The consequences of this research suggest that the chitosan, Moringaleaf powder, and their composites can be used to discover an antibacterial agent for developing new pharmaceuticals to control studied human pathogenic bacteria responsible for severe illness.Moringaoleiferais widely used in food and folk medicine; while chitosan is widely useful in food, detergents, textiles, leather, paper, pharmaceuticals, and cosmetics industries. Synergism/antagonism of Moringa-chitosan composites was based on concentrations on the tested organisms.

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Chitosan is regarded as a useful compound in medical and pharmaceutical technology. It is widely used in nanomedicine, biomedical engineering and development of new therapeutic drug delivery systems with enhanced bioavailability, specificity and reduced pharmacological toxicity attracting the attention of entrepreneurs, industrialists, academicians, environmentalists, medical scientist, and the general populace (Paul *et al*., 2018). Chitosan is a safe and friendly substance for human; therefore, it has become of great interest not only as an underutilized resource but also as a new functional material of high potential in various fields. Some unique properties make chitosan an excellent material for the development of new industrial applications and recent progress in chitosan material is quite noteworthy (Paul *et al*., 2018). Chitin and chitosan were attracted marked interest due to their biocompatibility, biodegradability, and non-toxicity (Hafsa *et al*., 2015). The need for new and effective anti-microbial agents with broad-spectrum of activity from natural sources is increasing day by day *(*Rahman *et al., 2008*). In recent years, there has been growing interest in research and development of new antimicrobial agents from various sources to combat microbial resistance (Mounyr *et al*., 2016). This study is indispensable as this natural biopolymer composites with *Moringa oleifera* leaf powder is been used on isolates promising to serve as a good antimicrobial agent to those microbes that are resistant to synthetic drugs with low cost without adverse effect on the human system. Hence, this study aimed at the synergetic/antagonistic antimicrobial potential of chitosan composites with *Moringaoliefera* leaf powder.

MATERIALS AND METHOD

*Sample Collection and Preparation:*The *Moringa oleifera leaf* was collected from Samaru Zaria, identified in herbarium section of Botany department of Ahmadu Bello University, Zaria washed and dried at room temperature to constant weight and macerated to powder while crayfish was bought from Samaru market in Zaria washed and dried in an oven to constant weight and grind to a powder. Commercial chitosan was purchased from Germany as standard.

*Synthesis of Chitosan (Maulin, 2017):*The sample (crayfish powder) was demineralized by soaking in

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10% HCl solution for 24 Hrs at 60ºC.Deproteinized by soaking in 10% NaOH solution for 24Hrs at 60ºC. And it was allowed to cool for 1 Hr. The solution was then filtered and then washed with demineralized water. After filtering the solution, the residue was washed with demineralized water and the process was followed by deacetylation by adding 50% NaOH for 8Hrs at 60ºC and washed with demineralized water to neutral pH.Commercial chitosan was obtained from Germany and compared with the synthesized chitosan.

Antimicrobial Activity of Chitosan Abdullahi et al., (2011): *Preparation of stock solution:* 200mg/ml of the compound was prepared by weighing 2g of the moringa powder and dissolved in 10ml of the solvent and serial dilution of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml were then prepared. While 200**µ**g/mlof chitosan was prepared by weighing 0.2g and dissolving in 10ml distilled water.and serial dilution of 100**µ**g/ml, **5**0mg/ml, 25**µ**g/ml, 12.5**µ**g/ml and 6.25**µ**g/ml were then prepared.

Preparation of Culture Media: This was done by weighing 38g of the medium (Mueller Hinton Agar) in one litre distilled water with frequent agitation and boiled to aid dissolution then autoclaved at 121ºC for 15 minutes and then allowed to cool to 45ºC and then 25ml of the sterilized medium was dispensed into sterilized Petri dishes and allowed to solidify.

*Standardization of Test Organisms:*Pure culture of the test microbes (*E. coli, S. typhi, S. Aureus, P. bulgaris* and *S. pneumonia*) were sub-cultured into normal saline and incubated at 37ºC for 24 Hrs. 0.5 McFarland turbidity standard was used to standardize the test microbes' suspension.

*Antimicrobial Susceptibility Test:*Agar well diffusion was used and 0.1ml standard inoculation of the test organism was uniformly streaked into freshly prepared Mueller Hinton Agar plate with the aid of a sterile swab stick using a sterile cork borer of 6mm diameter, two agar well were punched into each agar plate. 0.2ml of the compound's concentrations wereplaced in each well respectively and allowed to diffuse into the agar. The plates were incubated at 37ºC for 24 Hrs. The antimicrobial was expressed as the diameter of zone of inhibition produced by the compound and reported in millimeter (mm) *(*Abdullahi *et al.,* 2011).

*Minimum Inhibitory Concentration (MIC):*This was done using broth dilution method, 10ml of nutrient broth was dispensed into the test tube and sterilized at 121ºC for 15 minutes and allowed to cool. 0.1ml of the standard inoculums was dispensed into the broth medium. 0.1ml each of the compound serial dilution concentration was dispensed and the solution was evenly mixed and incubated at 37ºC for 24 Hrs. Test tubes with no turbidity were noted and the least concentration was reported as the MIC value *(*Abdullahi *et al.,* 2011).

Minimum Bactericidal Concentration (MBC): Freshly labeled sterile agar plates were used. The MIC test tubes of each test organisms were sub-cultured on sterile agar plates and then incubated at 37ºC for 24 Hrs. The compound serial concentration with no growth was noted and the MBC values reported. All results were compared with the standard antibiotic *(*Abdullahi*et al.,* 2011).

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 steps.There is a popular saying that "health is wealth" which is a very precious gift of life. Almost everybody takes it for granted until we are deprived of it as a result of sedentary life style (WHO, 2013).Synthetic drugs (antimicrobial drugs) are being used by patients as prescribed or not prescribed by physicians for treating microbial diseases.

Life-threatening invasive microbial infections are major problems in immune-compromised patients. Standard antimicrobial agents so far have been quite successful, but some of them have limited use due to toxicity, drug resistance and their clinical efficacy in some invasive microbial infections. The result obtained from this work indicated that chitosan, moringa leaf powder, and their composites at different ratios inhibited the growth of *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Proteus bulgaris and Streptococcus pneumonia* with varying diameter.

There was a synergistic effect at a higher ratio of chitosan to lower ratio of moringa leaf powder while the antagonistic effect was observed at a higher ratio of moringa powder to lower ratio of chitosan.The difference in antimicrobial properties of a plant might be attributable to the age of the plant used, freshness of plant materials, physical factors (temperature, light, water), contamination by field microbes, adulteration and substitution of plants, incorrect preparation and dosage (Calixto, 2000; Okigbo and Omodamiro, 2006; Okigbo and Igwe, 2007).

Table 1 The zones of inhibition of chitosan composites with *Moringa oleifera* leaf powder in mm

Test microbes	MD (mg/ml)					CD (µg/ml)					Composites (%)								
	200	100	50	25	12.5	200	100	50	25	12.5	1	2	3	4	5	6	7	8	9
S. aureus	+	++	++	++++	+++++	+	++	+++	++++	+++++	6.25	6.25	12.5	12.5	12.5	12.5	12.5	12.5	25
S. typhi	-	+	++	+++	+++	+	++	+++	++++	+++++	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
P. bulgaris	-	-	-	+	++	+	++	+++	++++	+++++	6.25	6.25	6.25	6.25	6.25	12.5	12.5	12.5	12.5
E. coli	-	-	+	++	+++	-	+	++	+++	++++	12.5	12.5	12.5	12.5	12.5	12.5	25	25	25
S. pneumonia	-	-	-	-	-	+	++	+++	++++	+++++	6.25	6.25	6.25	6.25	6.25	6.25	12.5	12.5	12.5

Key: *MD =Moringa in DMSO, CD=Chitosan in DMSO, 1=90%chitosan with 10% moringa, 2=80% chitosan with 20% moringa, 3=70% chitosan with 30% moringa, 4=60% chitosan with 40% moringa, 5=50% chitosan with 50% moringa, 6=40% chitosan with 60% moringa, 7=30% chitosan with 70% moringa, 8=20% chitosan with 80% moringa, 9=10% chitosan with 90% moringa,*

Table 2 The minimum inhibition concentration (MIC)

s/n	Test Organisms	MD	CD	1	2	3	4	5	6	7	8	9	Control Potency (µg/ml)	
1	*S. aureus*	11	10	18	16	14	15	12	11	11	11	11	Chloraphenicol 30	16
2	*S. typhi*	13	12	14	17	15	10	10	10	10	10	10	Chloraphenicol 30	17
3	*P. bulgaris*	19	11	22	25	21	17	15	12	8	8	8	Chloraphenicol 30	14
4	*E. coli*	15	14	15	14	13	13	11	10	10	10	10	Chloraphenicol 30	14
5	*S. pnuemoniae*	21	12	24	26	22	19	16	13	13	12	12	Chloraphenicol 30	12

Key: MD =Moringa in DMSO, CD = Chitosan in DMSO, 1=90%chitosan with 10% moringa, 2=80% chitosan with 20% moringa, 3=70% chitosan with 30% moringa, 4=60% chitosan with 40% moringa, 5=50% chitosan with 50% moringa, 6=40% chitosan with 60% moringa, 7=30% chitosan with 70% moringa, 8=20% chitosan with 80% moringa, 9=10% chitosan with 90% moringa,

Table 3 The minimum bactericidal concentration (MBC)

Test organism	MD (mg/ml)					CD (µg/ml)					Composites (%)								
	200	100	50	25	12.5	200	100	50	25	12.5	1	2	3	4	5	6	7	8	9
S. aureus	+	++	+++	++++	+++++	+	++	+++	++++	+++++	12.5	12.5	25	25	25	25	25	25	50
S. typhi	-	+	++	+++	+++	+	++	+++	++++	+++++	25	25	25	25	25	25	25	25	25
P. bulgaris	-	-	-	+	++	+	++	+++	++++	+++++	25	25	25	25	25	50	50	50	50
E. coli	-	-	+	++	+++	-	+	++	+++	++++	25	25	25	25	25	25	50	50	50
S. pneumonia	-	-	-	-	-	+	++	+++	++++	+++++	25	25	25	25	25	25	50	50	50

Key: MD =Moringa in DMSO, CD = Chitosan in DMSO, 1=90%chitosan with 10% moringa, 2=80% chitosan with 20% moringa, 3=70% chitosan with 30% moringa, 4=60% chitosan with 40% moringa, 5=50% chitosan with 50% moringa, 6=40% chitosan with 60% moringa, 7=30% chitosan with 70% moringa, 8=20% chitosan with 80% moringa, 9=10% chitosan with 90% moringa,

While the activity of chitosan is based on the concentration molecular weight, source, degree of deacetylation (Paul *et al*., 2018). Very wide zone of inhibition of chitosan composite with *Moringa* leaf powder showed that it had great potential as a remedy for infections/diseases caused by *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Proteus bulgaris, and Streptococcus pneumonia*. Chitosan is a promising material for biomedical as well as food science applications, it is a natural multifunctional polymer with unique and versatile properties. It is well-known for its significant biological and chemical properties. It is regarded as a useful compound in medical and pharmaceutical technology; widely used in nanomedicine, biomedical engineering and development of new therapeutic drug delivery systems with enhanced bioavailability, specificity and reduced pharmacological toxicity (Paul *et al*., 2018).

*Conclusion***:** The consequences of this research suggest that chitosan, Moringa leaf powder, and their composites can be used to discover an antibacterial agent for developing new pharmaceuticals to control studied human pathogenic bacteria responsible for severe illness. Extensive applications of chitosan and *Moringa* in pharmaceutics have been realized because they offer unique properties which so far have not been attained by many other materials.

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