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 vulgaris* 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, *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. *Moringa oleifera* is 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 (Ha'ssa 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 has 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 synergic/antagonic antimicrobial potential of chitosan composites with *Moringa oleifera* 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...
10% HCl solution for 24 Hrs at 60°C. Deproteinized by soaking in 10% NaOH solution for 24 Hrs at 60°C. 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 8 Hrs at 60°C 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: 200 mg/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 200µg/ml of chitosan was prepared by weighing 0.2g and dissolving in 10ml distilled water and serial dilution of 100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml and 6.25µg/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 121°C for 15 minutes and then allowed to cool to 45°C 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 37°C 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 were placed in each well respectively and allowed to diffuse into the agar. The plates were incubated at 37°C 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 121°C for 15 minutes and allowed to cool. 0.1 ml of the standard inoculum 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 37°C 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 37°C 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 lifestyle (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).
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 vulgaris, and Streptococcus pneumoniae. 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.

REFERENCES
Abdullahi, MI; Musa, AM; Haruna, AK; Sule, MI; Abdullahi, MS; Abdullmalik, MI; Akinwande, Y; Abimiku, AG; Iliya, I (2011). Antimicrobial Flavonoid Diglycosides from the leaves of ochnaschein furthiana (Ochnaceae). Nig. J. Pharm. Sci. 10 (2) 1-7.

Antony, RS; Manickam, TD; Saravanan, K; Karuppasamy, K; Balakumar, S (2013). “Synthesis, Spectroscopic and Catalytic Studies of Cu(II), Co(II) and Ni(II) Complexes Immobilized on Schiff base Modified Chitosan,” J. Mol. Structure, vol. 1050, pp. 53–60.
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Dutta, PK; Dutta, J; Tripathi, VS (2004). Chitin and Chitosan: Chemistry, Properties, and Applications. J. Sci and Indu. Res.63: 20-31.

Hafsa J; Smach, MA; Charfeddine B; Limem, K; Majdoub, H; Rouatbi, S (2015). Antioxidant and Antimicrobial Properties of Chitin and Chitosan Extracted from ParapenaeusLongirostris Shrimp Shell Waste. J.Pharma.

Maulin, S; Heet, C; Gaurav,D; Rushabh, P; Anjali, B; Sandeep, R(2017) Synthesis and Antimicrobial Properties of Chitosan

Paul, ED; Garba, ZN; James, DO (2018); Chitosan, A Natural Polymer for Health. ATBU, (JOSTE); Vol. 6 (4).

Rahman, MS; Rahman, MZ; Wahab, MA; Chowdhury, R; Rashid, MA (2008). Antimicrobial Activity of Some Indigenous Plants of Bangladesh. Dhaka University J. Pharma. Sci. 7: 23-26.

Roller, S; Covill, N (1999) “The Antifungal Properties of Chitosan in Laboratory Media and Apple Juice,” Int. J. Food Microb., vol. 47, no. 1-2, pp. 67-77.

Venter, JP; Kotze, AF; Auzely-Velty, R; Rinauda, M (2006); Synthesis and evaluation of mucoadhesivity of CD-chitosan derivative. Int. J. Pharma.313: 36-42.

World Health Organization, WHO (2013);WHO Proposes Survival for African Children

Younes, I; Rinaudo, M (2015). Chitin and Chitosan Preparation from Marine Sources Structure, Properties and Applications. Mar. Drugs 13: 1133-1174.