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Original Article

Bioactive Compounds and Nanoparticles Conjugated with Chitosan Composites for Clinical Purposes

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Abstract

Background: Various forms of microorganisms have developed multiple resistances against the effects of applied antibacterial agents controlling them. Hence, there is a need to formulate potent antimicrobial agents from natural sources to eliminate them from the environment. The aim of this study was to investigate the potency of chitosan conjugated with some medicinal plants against recalcitrant microorganisms and overcome the problem of multiple antibiotic resistance.

Methods: In this study, the synergistic effects of chitosan derived from snail shells were explored in conjunction with eight plant sources, including *Ocimum gratissimum*, *Croton zambesicus*, *Phyllanthus niruri*, *Aloe barbadensis*, *Moringa oleifera*, *Andrographis paniculate*, *Aloe barbadensis*, and *Curcuma longa*. The investigation focused on the antimicrobial properties of these combinations, aiming at enhancing the efficacy of pharmaceutical products against antibiotic-resistant strains. Four samples were analyzed in this regard. Sample A consisted of 0.5 g of sulphur nanoparticles (SNPs) conjugated with chitosan, and Sample B contained 1 g of SNPs conjugated with chitosan from shrimp shells dissolved in 1% acetic acid. In addition, Sample C involved chitosan from shrimp shells dissolved in 1% acetic acid, and Sample D utilized chitosan from snail shells dissolved in 1% acetic acid. All demonstrated varied antimicrobial potentials.

Results: Notably, Sample B, which utilized chitosan from shrimp shells as nanocarriers for SNPs, exhibited significant antimicrobial activity, with zones of inhibition measuring up to 35 mm and 37 mm against multidrug-resistant strains of *Staphylococcus* sp. (coagulase-negative), *Klebsiella oxytoca*, and *Escherichia coli*, respectively. Additionally, promising antimicrobial effects were observed against organisms such as *Pseudomonas aeruginosa*, *Klebsiella ornithinolytica*, and *E. coli*, with zone sizes reaching 20 mm, 23 mm, and 25 mm, respectively. Iron oxide NPs (Fe_3O_4) and silica-coated iron oxide NPs displayed lower activity levels, except at the 100 mg/mL concentration, where they represented efficacy against certain multidrug-resistant strains such as AKR 18 - *Enterobacter agglomerans*, OKI 10 - *Acinetobacter haemolyticus*, and T30 – *K. oxytoca*.

Conclusion: The findings of this study offer valuable insights into the management of infectious disease and the treatment of challenging pathogens in healthcare systems, providing a potential roadmap for combating antibiotic resistance and improving therapeutic strategies.

Keywords: Chitosan, Diseases, Nanocarriers, Nanoparticles

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Introduction

Nanoparticles (NPs) have become of interest to scientists based on some breakthroughs attained with regard to their use in disease detection and control, water purification, and other industrial applications. The Nobel Prize-winning American physicist, Richard Feynman, introduced the idea of nanotechnology. After fifteen years, Norio Taniguchi, a Japanese scientist, was the first to use and define the term "nanotechnology" in 1974 as "nanotechnology mainly consists of the processing of separation, consolidation, and deformation of materials by one atom or one molecule" (1).

In recent scientific research, chitosan has been conjugated with NPs as nanocarriers for some clinical and industrial purposes. Chitosan has recently become the most researched natural polymer (2), with commercial

applications in the biomedical, food, chemical, and pharmaceutical industries. Chitosan is a linear polysaccharide that is composed of and linked with some glucosamine. It is highly useful in everyday life for some domestic and various industrial purposes. It can be made by treating the chitin shells of crustaceans with alkaline substances such as sodium hydroxide. Chitosan is a safe and friendly substance for humans. It is useful in the adsorption, cosmetics, pharmaceutical, flocculant, anticancer, and antimicrobial industries. Chitosan can be synthesized from chitin extracted from crayfish. Methods such as deproteinization, demineralization, and deacetylation, respectively, can be used in the synthesis of chitosan from crayfish. It has good antimicrobial activity and, thus, can form an antibacterial synergistic composite with some plant sources. This is due to its unique characteristics as a naturally occurring, non-toxic, biodegradable, and biocompatible polymer with direct antibacterial action (3).

Chitosan's biological activities and commercial uses can be applied in the biomedical, food, and chemical industries (4). It has been shown to have antimicrobial action against various bacteria with a broad antibacterial spectrum, including gram-positive and gram-negative bacterial strains. *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Bacillus megaterium*, *Listeria monocytogenes*, *Lactobacillus brevis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, and *Salmonella typhimurium* were all found to be susceptible to chitosan (5). The multidrug-resistant nature of the different strains of microorganisms can be caused by various factors, including the formation of biofilms due to some environmental conditions as well as some clinical factors (6).

Rather than a single compound, chitosan [poly- (β-1/4)-2-amino-2-deoxy-D-glucopyranose] (7) is a group of partially or fully de-acetylated chitin molecules, a major component of the shells of crustaceans. Researchers are interested in chitosan due to some of its exceptional properties, such as biodegradability, biocompatibility, nontoxicity or low toxicity, non-antigenicity or low immunogenicity, and low-cost, as well as numerous antimicrobial, antitumor, antioxidant, antidiabetic, and immunoenhancing pharmacological properties (8). Chitosan has found wide applications in medicine, pharmacy, and food sciences (9).

Materials and Methods

Sample sources

The medicinal plant samples used for this study were collected from the wild and related sources in the Akungba-Akoko community and identified by experts in the Department of Plant Sciences and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

Sampling method

Collection and Preparation of Medicinal Plant Sources

There are hosts of medicinal plants, but some are commonly useful for resistant and recalcitrant microbes. Eight plant sources were used in this study, including *Ocimum gratissimum*, *Croton zambesicus*, *Phyllanthus niruri*, *Aloe barbadensis*, *Moringa oleifera*, *Andrographis paniculate*, *Aloe barbadensis*, and *Curcuma longa*.

The applied medicinal plants were obtained from the wild and Akungba-Akoko community, Ondo State, Nigeria. The leaves and roots of the plants were used, spread on clean plastic sheets, and allowed to air dry for 3 weeks in the laboratory. After complete drying, the individual parts were milled using the *Methods Optimization in Accelerated Solvent Extraction in Technical Note* (10) and stored in a plastic jar.

Extraction

Extraction processes were performed according to standard methods by the Association of Official Agricultural Chemists (11). About 5.00 g of the powdered samples were weighed and transferred to conical flasks and treated with 5 different solvents (petroleum ether, chloroform, butanol, ethanol, *N-hexane*, and water) until the powders were fully soaked. The flasks were shaken every hour for the first six hours, and then they were kept aside and shaken after 24 hours (12). The flasks were allowed to sit for three days, and then the extracts were filtered. The extracts were collected and evaporated to dryness using a rotary evaporator. A total of 30 extracts were obtained and carefully kept in airtight sample bottles prior to analysis.

Collection and Identification of Microorganisms

Thirty-five bacterial isolates recovered from the clinical specimens of patients in designated health facilities in Ondo State and related research sources were stored using nutrient agar medium at the University Health Centre as well as the Centre for Infectious Disease Research and Drug Development, Adekunle Ajasin University, Akungba-Akoko, Ondo State. The preliminary confirmatory test was earlier performed to confirm the identity of the organisms using the Microbact™ 24E (Oxford, UK) identification kit for this purpose (13).

Antibiotic Susceptibility Test

This test was conducted on bacterial isolates using a modified Kirby-Bauer's disc diffusion method on Muller-Hinton agar (MHA; CM337 – Oxoid, UK) (14). The isolates were subcultured into nutrient broth and incubated at 37 ˚C overnight (24 hours). The broth cultures were diluted with normal saline to the 0.5 McFarland turbidity standard. Appropriate inoculums were used accordingly. The MHA was incubated at 37 ˚C for 18 hours. The diameter of the zone of inhibition was measured and interpreted according to the Clinical Laboratory Standard Institute guidelines (15). Calibrated measuring rulers or

devices have been adopted for this purpose.

Antimicrobial Screening of Applied Medicinal Plant Part Extracts

The antimicrobial screening of the medicinal plant part extracts against some predetermined antibiotic-resistant clinical isolates was performed using the modified agar well diffusion method (14). A stock concentration of 100 mg/mL was constituted by dissolving 1 g of the extract in a 10 mL mixture of 30% dimethyl sulfoxide and sterile distilled water (1:3). The two-fold serial dilution of the stock was conducted to obtain varying concentrations of the extracts (50 mg/mL, 25 mg/mL, and 12.5 mg/mL). An aliquot of 1 mL of the standardized inoculum (0.5 McFarland turbidity standard) was mixed with 19 mL of molten MHA and left to solidify in a sterile Petri dish.

Using a sterile cork borer (6 mm in diameter), the wells were carefully made on the agar plate (at least 20 mm apart). In addition, 50 µL of each concentration of the extract and the controls were introduced into each well and left on the table to settle. The MHA plate was incubated at 37 ˚C for 20 hours, and the diameter of the zone of inhibition was measured. A potent antibiotic (Ofloxacin) and dimethyl sulfoxide were used as positive and negative controls, respectively.

A cork borer with a size of 6 mm was utilized, while for the control, water and ofloxacin served as negative and positive controls, respectively.

Preparation of Chitosan Samples

The synthesis of chitosan was based on the methods of previous studies (16,17). The sample (crayfish powder) can be demineralized by soaking in a 10% hydrogen chloride solution for 24 hours at 60 ˚C. Deproteinized by soaking in a 10% sodium hydroxide (NaOH) solution for 24 hours at 60 ˚C and allowed to cool for 1 hour. The solution was then filtered and 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 hours at 60 ˚C and washed with demineralized water to neutral pH. Commercial chitosan can be obtained from foreign countries such as Germany and the UK. Modified versions of the methods by Maulin et al (16) and Paul et al (17) were employed for the synthesis and extraction of chitosan from snail shells.

Sample Preparation

Maulin's method (16) was used for the synthesis and extraction of chitosan from two crustaceans, namely, crayfish and prawn.

Iron oxide (Fe_3O_4) NP used in this study is spherical and is a mineral also known as magnetite or magnetic oxide that is dark in color, ranging from a brownish hue to grey or black. The size of this particle, determined by the dynamic light scattering technique, helps measure random changes in the intensity scattered from the suspension/solution. This size can range from 0.3 to 10 000 nm. Fe_3O_4 NPs and silica-coated iron oxide NPs (Fe_3O_4 @Si) with plant extracts such as *Ocimum gratissimum* were tested as alternate antimicrobial agents against these resistant clinical strains.

Results

The multidrug-resistant bacterial isolates used in this study were identified by standard microbiological methods and included *Candida albicans*, *Budricia aquatic*, *Staphylococcus* sp. (coagulase –ve), *Klebsiella ornithinolytica*, *Escherichia coli*, *Staphylococcus aureus*, *Acinetobacter haemolyticus*, *Klebsiella ornithinolytica*, *Burkholderia cepacia*, *Pseudomonas aeruginosa*, *Escherichia hermannii*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Enterobacter gergoviae*, *Pseudomonas aeruginosa*, *Klebsiella terrigena*, *Klebsiella terrigena*, *Citrobacter gilleric* , *Klebsiella oxytoca*, and *Salmonella typhi* (Table 1). Similarly, the preparation flow chart of chitosan used for this research is shown in Figure 1.

The size of the applied Fe_3O_4 NP was 10.31 nm. Fe_3O_4 has a cubic inverse spinel group structure, which consists of a close-packed array of oxide ions where all the Fe_2^{2+}ions occupy half of the octahedral sites and Fe_3 is split evenly across the remaining octahedral sites and the tetrahedral sites. Various sample sources were used in this study, including Sample A, 0.5 g of SNPs together with chitosan, and Sample B: 1 g of SNPs together with chitosan from shrimp shell dissolved in 1% acetic acid. The other sources were Sample C: Chitosan from shrimp shell dissolved in 1% acetic acid and Sample D: Chitosan from snail shell dissolved in 1% acetic acid. They all showed diversified antimicrobial potentials in the test isolates (1).

Table 1 presents NPs synergized with chitosan as nanocarriers in Sample B with 1 g of SNPs together with chitosan from shrimp shell dissolved with 1% acetic acid, demonstrating high antimicrobial activity up to 35 mm and 37 mm zones of inhibition in *Staphylococcus* sp. (coagulase –ve), *Klebsiella oxytoca*, and *E. coli*, respectively.

Table 2 also provides the results related to the effectiveness of chitosan in synergy with some medicinal plants. It was observed that organisms such as *P. aeruginosa*, *K. ornithinolytica*, and *E. coli* with relatively high zone inhibition up to 20 mm and 23 mm susceptibility demonstrated the antimicrobial potential of the use of this biopolymer with selected medicinal plants. The observations made at this level can serve as a useful guide for the control of infectious diseases and the treatment of some recalcitrant etiologic agents in health management systems.

The antimicrobial potential of chitosan synthesized from snail and shrimp sources, respectively, was conjugated with *Aloe barbadensis* and *Curcuma longa* (Table 3). The use of *A. barbadensis* during this study shows low antimicrobial activity. Nevertheless, a combination of *A. barbadensis* and chitosan (from shrimp sources) revealed

Table 1. Chitosan Bioactive Activity with Nanoparticles on Clinical Isolates

Isolates	Zone of Inhibition in mm Unit			
	A	B	C	D
Candida albicans	-	-	-	-
Escherichia coli	14 (R.)	20	-	12
Budricia aquatic	-	14	12	11
Staphylococcus sp.	32	35	13	-
Escherichia coli	29	27	12	11
Escherichia coli	27	37	11	-
Klebsiella ornithinolytica	22	31	11	-
Escherichia coli	25	15	-	13
Escherichia coli	30	31	-	-
Escherichia coli	25	22	11	-
Staphylococcus aureus	-	-	-	-
Acinetobacter haemolyticus	23	26	-	12
Klebsiella ornithinolytica	29	32	-	11
Burkholderia cepacia	31	18	11	11
Pseudomonas aeruginosa	-	12	12	11
Escherichia hermannii	25	30	-	-
Staphylococcus aureus	27	21	-	-
Pseudomonas fluorescens	35	26	11	-
Pseudomonas fluorescens	35	20	10	12
Enterobacter gergoviae	-	32	-	21
Pseudomonas aeruginosa	-	20 (R.)	12	D
Klebsiella terrigena	26	27	19	12
Klebsiella terrigena	-	17 (B.S)	-	-
Citrobacter gilleric	27	25	-	-
Escherichia coli	17 (B.S)	15 (B.S)	-	-
Klebsiella oxytoca	27	26	-	-
Klebsiella oxytoca	20	19	-	-
Escherichia coli	20	28	-	-
Klebsiella oxytoca	25	35	12	11
Escherichia coli	31	12 (B.S)	-	-
Escherichia coli	30	25	13	-
Salmonella typhi	30	-	12	-

Legend:
Sample A: 0.5 g of SNPs together with chitosan.
Sample B: 1 g of SNPs together with chitosan.
The chitosan from the shrimp shell was dissolved in 1% acetic acid.
Sample C: Chitosan from shrimp shell dissolved in 1% acetic acid.
Sample D: Chitosan from snail shell dissolved in 1% acetic acid.

some antimicrobial potential, while chitosan (from shrimp sources) conjugated with *C. longa* had the highest antimicrobial potential against most multidrug-resistant clinical isolates tested in the present study (Table 3). Typical plant sources used for this purpose are shown in Figures 2–8, while Figure 10 displays applied antimicrobial susceptibility test cultures. Table 4 summarizes the results of the average minimum inhibitory and minimum bactericidal concentrations for selected isolates tested in this study.

The result of the use of sulphur NPs has been positive and encouraging. Other tested NPs, including Fe_3O_4 NPs and Fe_3O_4 @Si NPs, revealed relatively low activity, except at 100 mg/mL for some multidrug-resistant strains such as AKR 18 - *Enterobacter agglomerans*, OKI 10 - *Acinetobacter haemolyticus*, and T30 - *Klebsiella oxytoca*. Similarly, NPs combined with plant extracts such as *Ocimum gratissimum*, tested as alternate antimicrobial agents against these resistant clinical strains, partly, demonstrated relatively high activity, possibly due to some synergistic reactions (Table 5). .Generally, typical medicinal plant sources that their bioactive substances were tested for this research are shown in Figures 2 to 10.

Discussion

The emergence of multidrug-resistant microbes is globally worrisome. Thus, frantic scientific efforts have been made to conquer this resistant battalion. The findings of this study demonstrated the potential and complementary use of nanomaterials conjugated with chitosan in the control of multidrug-resistant and recalcitrant-etiologic agents (Tables 1 and 2). Nanomaterials are developed to exhibit novel physical, chemical, and biological characteristics that make them suitable for use in various applications, including the degradation of pollutants and wastewater treatment. The findings further highlighted the potency of the nanomaterials used for biocontrol purposes, which corroborates with the results of a study by Rezaei et al (18), confirming the benefits of the use of NPs in the control of diseases and some damages that can be incurred in the process if care is not taken.

Nanomaterials are also used as nanofilters for water purification. Similarly, they are valuable for pollutant sensing, which is another important application of microbial nanotechnology in nanocatalysis. The application of nanomaterials enumerated here corroborates the research and development efforts at the atomic or molecular level to create structures and systems applicable in diverse aspects, as reported by Drexler et al (19) as well as Balzani (20). This nanotechnology approach, with the complementary use of chitosan, covers various fields of the medical, pharmaceutical, clinical, and food industries (5).

Nanomaterials can be grouped into four major types, including inorganic-based, carbon-based, organic-based, and composite-based nanomaterials. However, SNPs prepared by sodium thiosulphate and hydrochloric acid capped with chitosan in this study showed diversified antimicrobial potentials on test isolates. The results of this study also conform to those of the study by Kalia et al (21), demonstrating the potential inhibitor for quorum sensing-controlled virulence factors and biofilm formation in *P. aeruginosa*, which helps overcome the multidrug-resistant nature of this kind. As intensified in this study, SNPs are more effective than Fe_3O_4 NPs and Fe_3O_4 @Si NPs that represented relatively low activity, except at 100 mg/mL for some multidrug-resistant strains such as AKR 18 - *Enterobacter agglomerans*, OKI 10 - *Acinetobacter haemolyticus*, and T30 - *Klebsiella oxytoca*. Similarly, NPs combined with plant extracts such as

Table 2. Chitosan Conjugated With Selected Medicinal Plants

Isolates	(Zone of Inhibition in mm)									
	A	B	C	D	E	F	G	H	I	J
Candida albicans										12
Budricia aquatic										
Staphylococcus sp. (coagulase –ve)						10	10	11		20 BS
Escherichia coli						11 BS				
Escherichia coli						10 BS		12		15 BS
Klebsiella ornithinolytica	11									
Escherichia coli										
Escherichia coli						10	10		11	15
Escherichia coli						9	11BS		9BS	13
Staphylococcus aureus	12	11BS								
Acinetobacter haemolyticus						12	10	18BS	11	
Klebsiella ornithinolytica						10BS		20BS		10
Burkholderia cepacia										14
Pseudomonas aeruginosa						13BS	10BS			11BS
Escherichia hermannii	11		10BS							
Staphylococcus aureus		10	12	19	10	13	9		10	18
Pseudo fluorescens	11		12						10	14BS
Pseudomonas fluorescens	12		15BS							15
Enterobacter gergoviae										
Pseudomonas aeruginosa										23BS
Klebsiella terrigena										
Klebsiella terrigena							11		10	15
Citrobacter gilleric	11BS	10	11		10					
Escherichia coli			11						10BS	
Klebsiella oxytoca						10BS				15
Klebsiella oxytoca										
Escherichia coli								15BS		
Klebsiella oxytoca	17BS		13BS							
Escherichia coli							10			11
Escherichia coli						11				25
Salmonella typhi								15BS		11

Specimen Number
Ocimum gratissimum (large) ethanol- A BOA
Ocimum gratissimum (small) ethanol- B SOB
Croton zambesicus ethanol- C CZC
Phyllanthus niruri ethanol -D PND
Moringa oleifera ethanol -E MOE
Ocimum gratissimum (large) N-hexane – F BOF
Ocimum gratissimum (small) N-hexane – G SOG
Croton zambesicus N-hexane -H CZH
Phyllanthus niruri N-hexane – I PNI
Andrographis paniculata ethanol -J APJ
BS - Bacteriostatic

Ocimum gratissimum tested as alternate antimicrobial agents against these resistant clinical strains partly revealed relatively high activity, possibly due to some synergistic reactions (Table 5).

To this end, the synergistic impact of the use of nanomaterials has intensified clinical specificity in this research and diversified applications, as reported by Upadhayay et al (22); they demonstrated the synergistic impact of nanomaterials and plant probiotics in agriculture. This study enhances measures to safeguard health through the adaptation of an appropriate nutritional approach. Further work is still ongoing on green NP synthesis.

The complementary use of chitosan, which is a biopolymer as a nanocarrier, is found valuable for its ability to reduce infectivity when conjugated and synergized with some potent medicinal plants. However, pharmacological standardization and clinical evaluation of medicinal plants are essential to making the natural products discussed

Table 3. Chitosan Conjugated with *Aloe barbadensis* and *Curcuma longa*

Isolates	(Zone of Inhibition in mm)		
	A	B	C
Owo 15 - Pseudomonas aeruginosa	11 mm	-	15 mm
IK 24 - Escherichia coli	-	-	-
T 32 - Escherichia coli	-	-	-
Owo 6 - Klebsiella terrigena	-	-	12 mm
Owo 5 - Pseudomonas aeruginosa	-	-	-
T11 - Citrobacter gilleric	-	12 mm	14 mm
IK 13 - Klebsiella ornithinolytica	-	14 mm	13 mm
Owo 28 - Escherichia coli	-	-	-
Owo 16 - Escherichia hermannii	-	-	13 mm
IK 27 - Budricia aquatic	-	14 mm	12 mm
T 29 - Escherichia coli	-	-	-
Owo 9 - Klebsiella terrigena	-	-	-
T 12 - Budricia aquatica	-	-	-
OKI 26 - Staphylococcus spp.	-	-	-
OKI 10 - Acinetobacter haemolyticus	-	-	-
OKI 18 - Enterobacter agglomerans	-	-	25 mm
IK 9 - Escherichia coli	-	-	-
T 28 - Escherichia coli	-	-	-
T 23- Klebsiella oxytoca	-	-	16 mm
AKR 18 - Enterobacter agglomerans	-	-	14 mm
AKR 17 - Escherichia coli	-	-	-
AKR 7 - Staphylococcus sp. (coagulase –ve)	-	-	14 mm
AKR 16 - Escherichia coli	-	-	12 mm
IK 14 - Escherichia coli	-	-	12 mm
OKI 05 - Staphylococcus aureus	-	-	11 mm
T 30 - Klebsiella oxytoca	-	-	-
Owo 30 - Enterobacter gergoviae	-	-	-
Owo 13 - Burkholderia cepacia	-	-	-
Owo 22 - Pseudomonas fluorescens	-	12 mm	15 mm

Specimen Number
A = *Aloe barbadensis* + Chitosan (from snail shell) ABA
B = *Aloe barbadensis* + Chitosan (from shrimp) ABB
C = *Curcuma longa* + Chitosan (from shrimp) CLC

in this context standard remedies that could effectively combat some mutated pathogens that have developed resistance against antibiotic abuse. The study of chitosan has proven its use to be valuable for clinical applications. It can also be useful for some industrial purposes, including animal feed supplements (23).

Chitosan-based biomaterials have been used in various biomedical and industrial processes, including drug tissue engineering, wound healing, regenerative medicine, blood anticoagulation, bone, tendon, or blood vessel engineering, dentistry, biotechnology, biosensing, cosmetics, water treatment, agriculture, and vaccine systems (24).

Chitosan composites have been conjugated with bioactive compounds and NPs for clinical purposes. For instance, chitosan-based composites have been employed in the preservation of meat and meat products, postharvest foods, and monitoring freshness/spoilage. In the biomedical industry, chitosan-based composites have been utilized in drug delivery, tissue engineering, and wound healing (24,25).

The antimicrobial properties of chitosan-based composites have undergone extensive investigation. Chitosan has been shown to have significant antimicrobial activity against a wide variety of fungi and bacteria. However, the mechanism of action for its antimicrobial activity is not yet fully understood, and further research is needed to establish a consensus. Chitosan-based (nano) materials have also attracted significant attention in the biomedical field due to their unique biocompatible, non-toxic, and antimicrobial nature. Chitosan-based nanomaterials have been used as antimicrobial wound dressings, brain drug delivery carriers, and in other biomedical applications (26,27).

Chitosan-based composites have been utilized in the preservation of food products by incorporating plant extracts to enhance their antimicrobial and antioxidant properties. This approach has been shown to improve the shelf life and quality of food products while minimizing the use of plastic material (28,29).

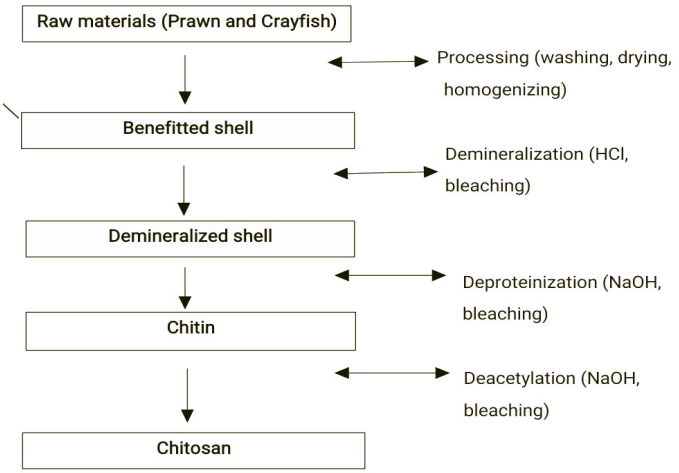

Figure 1. Flowchart for Chitosan Preparation. Sample A: 0.5 g of sulphur NPs (SNPs) together with chitosan. Sample B: 1 g of SNPs together with chitosan. The chitosan from the shrimp shell was dissolved in 1% acetic acid. Sample C: Chitosan from shrimp shell dissolved in 1% acetic acid. Sample D: Chitosan from snail shell dissolved in 1% acetic acid. Other tested NPs.

Figure 2. Typical Plant Sources: *Phyllanthus niruri*

Figure 3. Aloe vera

Figure 4. *Croton zambesicus*

Figure 5. *Moringa Oleifera*

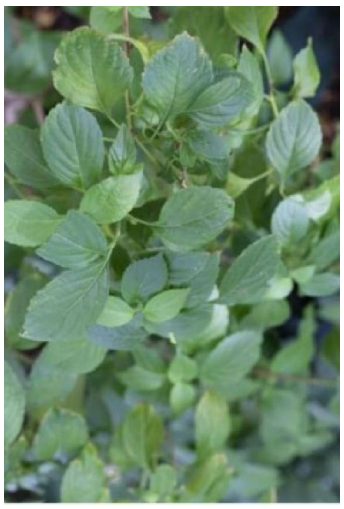

Figure 6. *Ocimum gratissimum*

Figure 7. *Ocimum gratissimum*

Figure 8. *Andrographis paniculata*

Figure 9. *Curcuma longa*

Figure 10. Cultures of Bacterial Strains Showing Zones of Inhibition Against Applied Samples

Table 4. Average MIC and MBC for Selected Isolates Tested

Name	Control	12.5	25	50	100
Staphylococcus aureus	29	-	11	13	21
Bacillus cereus	24	22	22	24	26
Escherichia coli	15	12	14	14	15
Salmonella typhi	22	-	-	-	14
Proteus mirabilis	22	9 (BS)	9.5 (BS)	11 (BS)	12 (BS)
Pseudomonas aeruginosa	15	-	-	11 (BS)	11
Salmonella pullorum	18	-	-	20	22

Note. MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; Ofloxacin was used as the standard antibiotics control.

Table 5. Iron Oxide Nanoparticles Tested Cultures

Bacterial Isolates	Zone of Inhibition in (MM)				
Lab code	**A**	**B**	**C**	**D**	**E - Control**
AKR 18 - *Enterobacter agglomerans*	25	-	-	-	25
T18 - *Klebsiella oxytoca*	-	**32**	**33**	-	30
OKI 10 - *Acinetobacter haemolyticus*	22	-	-	-	-
T30 - *Klebsiella oxytoca*	24	**34**	-	-	32

Key: A – Fe_3O_4 NPS (50%); B – Fe_3O_4 NPS (100%); C – Fe_3O_4 NPS + *Ocimum gratissimum* L.; D – Fe_3O_4 @Si; E – Control, Ofloxacin; BS – Bacteriostatic.
Note. MIC: Minimum inhibitory concentration. The average MIC for *Andrographis paniculata*, which is among the most potent antimicrobial agents against multidrug-resistant strains, and the mostly tested plant extract is 25 mg/mL, while the MBC stands at 50 mg/mL.

In summary, chitosan composites have shown great potential in clinical applications due to their unique properties and versatility. Further research is required to fully understand the mechanisms of action and to expand their applications in various fields.

Conclusion

The complementary use of Chitosan which is a biopolymer as nanocarrier is found valuable for its ability to reduce infectivity when conjugated and synergized with some potent medicinal plants. However, pharmacological standardization and clinical evaluation on medicinal plants are essential to make the natural products discussed in this context to be standard remedies that could effectively combat some mutated pathogens that have developed resistance against antibiotics abuse. Study of chitosan has proven its use to be valuable for clinical applications. It can also be useful for some industrial purposes including animal feed supplements. Use of some nanomaterials in biosensors can be much valuable in diseases detection. This research work therefore provide a valuable database that can be adopted by health management systems.

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Authors' Contribution

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Competing Interests

There is no conflict of interests.

Ethical Approval

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