Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/xxx
DOI URL: http://dx.doi.org/10.21474/IJAR01/13571

RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY OF CHITOSAN, *MORINGA OLEIFERA*, *CROTON ZAMBESICUS* AND THEIR SYNERGISTIC ACTIVITY AS IMMUNE BOOSTER

Olajide A. Ajayi[1,4], Goshen D. Miteu[2,4] and Blessing C. Ezeh[3,4]

1. Adekunle Ajasin University, Department of Microbiology. Centre for Infectious Disease Control and Drug Development (CIDCDD), AAU, Akungba-Akoko, Ondo State, Nigeria.
2. Caleb University, Imota, Lagos, Department of Biochemistry. Centre for Infectious Diseases Control and Drug Development (CIDCDD), AAU, Akungba-Akoko, Ondo State, Nigeria.
3. Federal Polytechnic Bauchi, Department of Chemistry/Biochemistry.
4. Centre for Infectious Diseases Control and Drug Development (CIDCDD), AdekunleAjasin University, Akungba-Akoko, Ondo State, Nigeria.

Manuscript Info

Manuscript History
Received: 24 August 2021
Final Accepted: 27 September 2021
Published: October 2021
Key words:-
Antimicrobial, Ethno-Medicinal, Immune Booster, Synergy

Abstract

The plants used for this study were selected from a collection of medicinal herbs collected from traditional healers during a prior ethnobotanical investigation. Chitosan derived from chitin is effective as an antibacterial agent in pharmaceutics. The Mauline, 2017 approach, which incorporates processing/maceration, demineralization, deproteinization, and deacetylation, was used to synthesize chitosan from crayfish and prawns. *Moringa oleifera* and *Croton zambesicus* were chosen for their phytochemical capabilities, including antibacterial, anti-inflammatory, and antioxidant properties. These plants were acquired locally, and combined with the various chitosan extracts in powdered form. They were tested against selected clinically resistant bacterial and fungal isolates. Chitosan extracted from crayfish and the synergized extracts of Chitosan sources with *Croton zambesicus* were effective against organisms like *Candida albicans*, *Vibrio cholerae*, *Enterobacter cloacae* and *Salmonella typhi* with 30mm, 13mm and 12mm zones of inhibition respectively. Water and diethylether extracts of *Croton zambesicus*, synergized with one (1%) Chitosan extracted from crayfish were only effective against *Bacillus cereus* and *Eschericha coli*. During the experimental stages, both bacteriostatic and bacteriocidal effects were observed in some media cultures; however, the overall results from the synergy between Chitosan (crayfish and prawn extracts), Moringa, and *Croton zambesicus* shows the possibilities of exploring these combinations in novel drug designs.

Introduction

Human health and the environment are inextricably linked. If the body is unfit owing to a weakened immune system (deficiency), infectious microorganisms such as viruses, bacteria, and fungi may readily attack, resulting in the emergence of numerous illnesses. Antibiotics or other synthetic medication treatments are often used to treat

Principal Investigator:- Olajide A. Ajayi
Corresponding Author:- Goshen D. Miteu
Address**:** Adekunle Ajasin University, Department of Microbiology. Centre for Infectious Disease Control and Drug Development (CIDCDD), AAU, Akungba-Akoko, Ondo State, Nigeria.

developing diseases in order to achieve an immediate healing effect. Another issue that will arise as a result of this practice is antibiotic and synthetic drug resistance. Furthermore, some medicines may cause adverse effects such as nausea, bone marrow destruction, thrombocytopenic purpura, and agranulocytosis, which can lead to other illnesses, necessitating the development of new therapies derived from natural sources (medicinal plants) (Sharififar *et al*., 2009). Modulating the immune system may help to prevent illnesses and free radicals from attacking the body. An immunomodulator is a substance that is able to modulate the function and activity of the immune system (Koruthu *et al*., 2011).

There is a dearth of information on the synergistic effect of these plants and their utility as an immune booster and in medication development. Antibiotic drug resistance in bacteria has forced a quest for new antimicrobial compounds from other sources, such as animal extracts and medicinal plants. Bacterial mechanisms have evolved their genetic capacity to gain drug resistance, particularly those used as therapeutic agents in the medical sector. For example, as many studies have shown, active efflux is a resistance mechanism evolved by most bacteria species against practically all antibiotics. Non-drug specific proteins, which make up the bulk of efflux systems, may recognize and export a wide range of chemically and structurally unrelated bacterial molecules without causing drug breakdown or modification (Kumar and Schweizer, 2005).

Chitosan is a biopolymer made from chitin, found in the exoskeletons of prawns, crabs, shrimp, lobsters, and crayfish. In the environment, these crustaceans can be found in both fresh and saltwater basins. Deacetylation of chitin in any of these crustaceans results in chitosan, a fibrous material. This fibre has strong antibacterial properties and can treat a wide range of microorganisms (Paul *et al*., 2019). Previous research has demonstrated that chitosan, with or without chemical modification, can be effective against microorganisms such as fungi and bacteria (Phaechamud, 2008).

Chitosan's antibacterial and anticancer characteristics are due to its structure, which encapsulates amine/acetamido and hydroxyl groups at 2, 3 and 6 carbon positions, respectively, in repeating units. *In vivo* and *In vitro*, chitosan's antibacterial action is efficient against many microorganisms (Rabea *et al*., 2003; Ganan *et al*., 2009). Chitosan can be used alone as an antibacterial agent (Islam *et al*., 2011). Also, it can be combined with ethnomedicinal plants to boost its antimicrobial effectiveness and use (Batista *et al*., 2003). Chitosan is available in three different types: alpha, beta, and gamma. The most common are alpha and beta, which have distinct molecular weights. The antibacterial activity of chitosan is influenced by the type of microbe, ambient circumstances, and the type of chitosan used (Hafdani *et al*., 2011). Its poly-cationic characteristics can explain the antibacterial mechanism of chitosan. Chitosan's antimicrobial action method is based on the fact that it interacts with the pathogenic membrane's negatively charged phospholipids and inhibits its activity. It acts as a chelating agent, penetrates the cell wall to bind to DNA, changes membrane permeability, and leaks intracellular contents (Rabea *et al*., 2003; Goy *et al*., 2009), resulting in cell death as a result of cell lysis caused by electrostatic interaction between positively charged chitosan sites and negatively charged microbial cell membrane (Rabea *et al*., 2003; Goy *et al*. (Tripathi *et al*., 2008).

There is also a probability that the charged chitosan will interact with some essential minerals, reducing their potency and inhibiting microbial growth (Jia *et al*., 2001). Chitosan's antibacterial efficacy improves as its concentration rises (Goy *et al*., 2016; Tayel *et al*., 2010).

The Moringa plant is grown for its leaves, fruits, roots, and seeds, which are used for various purposes, including food and medicine. Almost every component of the plant has nutritional value. The leaves and pods, on the other hand, are more commonly utilized as food or supplements. *M. oleifera's* young leaves are edible and are used in traditional diets in many areas where the tree grows. They are cooked or used as dried leaf powder in meals. Peanuts are made from seeds, and the oil is also edible. The treatment and prevention of malnutrition, particularly in children, is one of the most well-known applications of Moringa leaf powder. *M. oleifera* has a long history of medical usage in folklore. Plant parts other than the leaves, particularly the roots and seeds, are responsible for most of the plant's therapeutic applications.

Moringa oleifera Lam. is one of the species which is known for having the immunomodulatory activity to the immune system (Ugboko *et al*., 2020; Gupta *et al*., 2010). This plant has a high value because almost all parts of the plant (leaf, roots, stems, flowers, fruit peel and seeds) can be used as highly nutritious food and also has been reported to have antimicrobial compound (Moyo *et al*., 2012). This plant also serves as an immune system builder and is used in some countries to overcome malnutrition and malaria (Thilza *et al*., 2010). This herb was chosen due to its rich phytochemical compound, which including saponins, carotenoids, phenolic compounds and flavonoids.

Saponin and flavonoid can serve as natural immunomodulator that is expected to enhance lymphocytes cell development which is very important in the immune system (Wagner, 1999).

Croton zambesicus is is a medicinal plant that is primarily found in Africa. It is known by many names depending on where it is found. It is commonly known as "Ajekobale" in Ondo State, Nigeria, among other names. The leaves and twigs of the plant are used to extract the plant's therapeutic powers. The leaf decoction is used as an anti-hypertensive and antimicrobial (urinary infections) remedy in Benin (Adjanohoun *et al*., 1989) and as an anti-diabetic and malarial remedy in portions of Nigeria (Adjanohoun *et al*., 1989). The Ibibios of Nigeria's Niger Delta region utilizes the roots as an antimalarial, febrifuge, and anti-diabetic (Boyle *et al*., 2007). The root is also used in Sudan for menstruation pain and as aperients (El-Hamidi, 1970). Ngadjui *et al*., (1999) and Boyom *et al*., (2002) investigated the composition of essential oils extracted from *Croton zambesicus* leaves, stems, and roots and discovered that the three types of oils were comparable in composition, with monoterpenes abundant in the leaves and stems and sesquiterpenes in the root bark. Spathulenol and linalool were revealed to be critical components of the root and stem bark oils, which were shown to be rich in oxygen-containing chemicals. Antiplasmodial, anti-diabetic, anti-inflammatory, analgesic, and antipyretic activities have been reported for the ethanolic leaf extract (Boyle *et al*., 2007; Mcnulty, 2007), while the root extract has been reported to have antimalarial, anticonvulsant, and antiulcer activities. Ent-trachylobanediterpene, derived from dichloromethane extract of the leaves, shows the cytotoxic effect on Hela cells, according to Block *et al*., (2002). Antimicrobial properties of the leaf and stem have also been studied (Abo *et al*., 1999).

Ethno-medicinal surveys in Africa (Nigeria) have found that various plants can have beneficial therapeutic effects. *Moringa oleifera* and *Croton zambesicus* are among the two frequently cultivated and utilized plants that can be found in our habitats. Folk medicine practitioners and the Nigerian ethnomedicine for microbiological infections, anti-inflammation, sexually transmitted diseases, malnutrition, and diarrhoea have also recognized the efficacy of these herbs in treating a variety of ailments. This study aims to extract, synthesize, and evaluate the antibacterial potential of *Moringa oleifera*, *Croton zambesicus*, and chitosan from crayfish in synergy to assess their antimicrobial efficacy and utility as an immune booster.

Materials and Methods

Sample preparation

Maulin's method (2017) was used for the synthesis and extraction of chitosan from two crustaceans: crayfish and prawn.

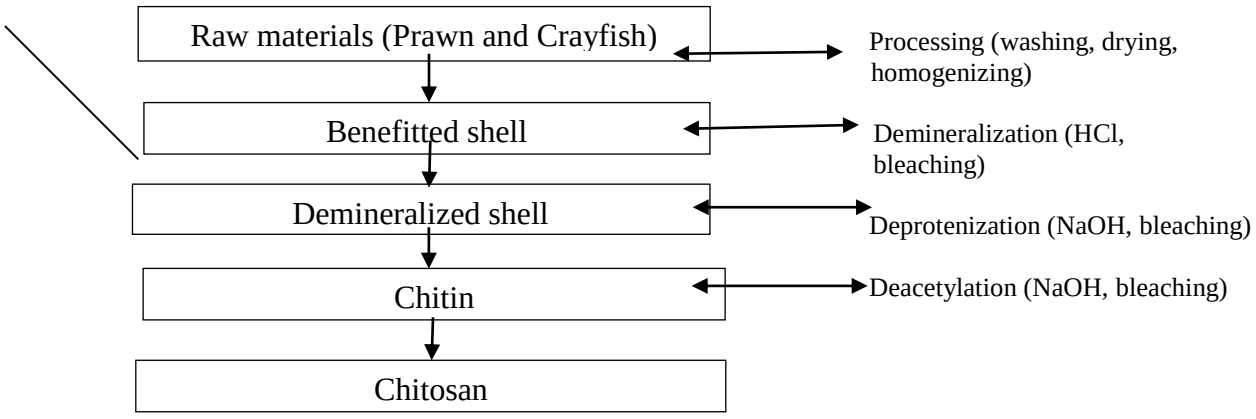

Fig 1: Flowchart for chitosan preparation.

Plant preparation and extraction

Moringa oleifera and *Croton zambesicus* were acquired from the local market at Akungba-Akoko, Ondo State, Nigeria, and authenticated. The leaves were washed, dried and macerated to their powder form.

0.5g of *Croton zambesicus*, Moringa and chitosan powder/fibre (from crayfish and prawn) were weighed and solubilized in water. The extracts were diluted with DMSO and water in a ratio of 3:1. Moringa powder and chitosan from prawn was diluted with 5mL of water, while *Croton zambesicus* and chitosan extracted from crayfish were diluted with 7mL of water. The dilution factor is vital in order to get more extracts for the antimicrobial analysis.

Procedures for antibiotic susceptibilities screening

Cultures of *Salmonella typhi*, *Pseudomonas aeraginosa*, *Enterobacter cloacae*, *Klebsiella oxytoca*, *Vibrio cholerae*, *Staphylococcus aureus*, and *Candida albicans* were used for this study. The plates were sterilized with an autoclave and divided into two groups; the first group was used to study the individual antimicrobial effect of the sample with these micro-organisms, while the second group was used to evaluate the synergistic antimicrobial effect of the samples on the micro-organism.

Mueller Hinton Agar (MHA) was prepared according to manufacturer's specification and sterilised to evaluate microbial growth and antimicrobial potential of the bacterial isolates. Using the direct pouring method, MHA was poured into the Petri dishes, was allowed to solidify, and then a cork borer with a diameter of 9mm was used to bore holes on the Petri plates. Five holes were bored, and the center was used as the control using a modified CLSI, (2016) method.

Broth culture needed for proper growth of the isolates was also prepared (3:10 w/v) – this is to enable the micro-organism viable in an artificial environment and evaluate its reaction and sensitivity. The prepared broth was poured into 7 test tubes at an equal volume of 9mL, respectively, sterilized for 15 minutes using an autoclave. After sterilization, the broth was allowed to cool. After that, each test tubes containing the broth was inoculated with 0.5mL broth containing viable micro-organisms taken from an already prepared broth culture. The test tubes were labelled correctly accordingly, and then the streak method was used to introduce each of the micro-organisms in respective plates.

The antibiotics, tetracycline was used as the positive control. Tetracycline is water-soluble; therefore, it was diluted with water to increase its surface area for better sensitivity. The syringe was used to introduce the control and the extracts into the plates.

Plant extracts and their antimicrobial activities

The Agar disc diffusion method (Mwitari *et al*., 2013, CLSI 2016) was used to test the ability of the various extracts to inhibit bacterial growth. Eight samples highlighted below were used as antimicrobial analytic screening purposes.

Sample A	*Moringa oleifera*
Sample B	*Croton zambesicus*
Sample C	Chitosan (Crayfish)
Sample D	Chitosan (Prawn)
Sample E	*Moringa oleifera* + Chitosan (Crayfish)
Sample F	*Moringa oleifera* + Chitosan (Prawn)
Sample G	*Croton zambesicus* + Chitosan (Crayfish)
Sample H	*Croton zambesicus* + Chitosan (Prawn)

Two different replicates of antimicrobial controls were used for each comparative analysis. A positive control named tetracycline was used in the microbial activities presented in first set of the assay, while water was used as a negative control for microbial activities presented in second set of the assay.

In the first antimicrobial analysis, the dilution ratio in 100mg/mL were; A: 0.09g of Chitosan from crayfish diluted in 0.9mL of solvent plus 1mL of *Croton zambesicus* diethylether extracts. B: 1g of Chitosan from prawn diluted in 10mL of solvent plus 1mL of *Croton zambesicus* water extracts. C: 0.09g of Chitosan from crayfish diluted in 0.9mL of solvent plus 1mL of *Croton zambesicus* diethylether extracts. D: 1g of Chitosan from prawn diluted in 10mL of solvent plus 1mL of *Croton zambesicus* water extract.

In the second phase, the dilution ratio in 100mg/mL were:

A: 1g of *Moringa oleifera* (diethylether extracts) was dissolved in 10mL solvent containing 2.5mL of DMSO and 7.5mL of water. B: 1g of *Moringa oleifera* (water extracts) was dissolved in 10mL solvent containing 2.5mL of DMSO and 7.5mL of water. C: 0.25g of *Croton zambesicus* (diethylether extract) was dissolved in 2.5mL of solvent containing 0.6mL of DMSO and 1.9 mL of water. D: 0.25 of *Croton zambesicus* (water extracts) was dissolved in 2.5mL of solvent containing 0.6mL of DMSO and 1.9mL of water. One (1%) percent, that 0.1mL of Chitosan from crayfish was added to the 10mL prepared extract dissolved with DMSO to synergize it.

Results

This study shows the antimicrobial potential and synergistic properties of *Croton zambesicus*, *Moringa oleifera* and Chitosan. Table 1 shows the results of antimicrobial assay studying synergistic reaction of water extracts of *Croton*

zambesicus, *Moringa oleifera* and Chitosan synthesized from both crayfish and prawn. Chitosan extracted from crayfish and the synergized extracts of Chitosan sources with *Croton zambesicus* were effective against organisms like *Candida albicans*, *Vibrio cholera*, *Enterobacter cloacae* and *Salmonella typhi* with 30mm, 13mm and 12mm zones of inhibition respectively. In Table 2, water and diethylether extracts of *Croton zambesicus*, synergized with one (1%) Chitosan extracted from crayfish were only effective against *Bacillus cereus* and *Escherichia coli*.

Table 1: Synergistic reaction of water extracts of *Croton zambesicus*, *Moringa oleifera* and Chitosan synthesized from crayfish and prawn.

Name of organism	Disc concentration of extract (mLs) (synergy mixture)	Interpretive categories of zone diameter in the nearest whole mm A	B	C	D	E	F	G	H
Candida albicans	0.1	-	-	30	-	-	-	30	-
Pseudomonas aureginosa	0.2	-	-	-	-	-	-	-	-
Klebsiella oxytoca	0.1	-	-	-	-	-	-	-	11
*Vibrio cholera*e	0.1	-	-	13	-	-	11	11	-
Enterobacter cloacae	0.2	-	-	13	-	11	-	-	11
Staphylococcus aureus	0.2	-	-	-	-	-	-	11	-
Salmonella typhi	0.1	-	-	-	-	11	-	12	12

Legend:

Sample A *Moringa oleifera*
Sample B *Croton zambesicus*
Sample C Chitosan (Crayfish)
Sample D Chitosan (Prawn)
Sample E *Moringa oleifera* + Chitosan (Crayfish)
Sample F *Moringa oleifera* + Chitosan (Prawn)
Sample G *Croton zambesicus* + Chitosan (Crayfish)
Sample H *Croton zambesicus* + Chitosan (Prawn)

Table 2: Synergistic reaction of *Croton zambesicus*, *Moringa oleifera* extracted with Diethylether solvent and chitosan synthesized from both crayfish.

Name of organisms	Disc concentration of extract (mLs) (synergy mixture)	Interpretive categories and zone diameter in nearest whole mm A	B	C	D
Staphylococcus aureus	0.1	-	-	-	-
Acinetobacter baumami	0.1	-	-	-	-
Klebsiella pneumonia	0.1	-	-	-	-
Bacillus cereus	0.1	17	12	-	-
Escherichia coli	0.1	20	11	-	-
Salmonella typhi	0.2	-	-	-	-
Proteus mirabilis	0.2	-	-	-	-
Pseudomonas aeruginosa	0.1	-	-	-	-
Salmonella pullorum	0.2	-	-	-	-
Candida albicans	0.1	-	-	-	-

Legend:
A: *Croton zambesicus* (Diethylether extract) + 1% Chitosan
B: *Croton zambesicus* (Water extract) + 1% Chitosan
C: *Moringa oleifera* (Diethylether extract) + 1% Chitosan
D: *Moringa oleifera* (Water extract) + 1% Chitosan

The diameter of zone around each disc (external diameters of visible zones of growth inhibition) were measured and recorded as described in numerous studies (Mwitari *et al*., 2013; Gupta *et al*., 2010; Moyo, 2012;). The average of the triplicate zone tests was calculated. The degree of activity of the seven samples was classified according to inhibition zone diameter as presented by Mwitari *et al*. (2013) with some modification; no inhibition: <7 mm; low inhibition: 7 - 7.9 mm; active inhibition: 8 -11 mm; highly active inhibition: 11.1 – 12mm highly active; and very active inhibition: >12 mm.

Discussion:

The antimicrobial assay from this experiment shows promising result of Chitosan synergism with selected medicinal plants. Chitosan extracted from crayfish showed a high antimicrobial activity against *Candida albicans*, *Vibrio cholera*e, *Enterobacter cloacae* which is in line with studies carried out by Sabaa *et al.,* (2018) and Pei *et al*., (2019). Just like the study carried out by James *et al*., (2019), the synergy between *Moringa oleifera* water extracts and Chitosan extracted from Crayfish has activity against *Vibrio cholerae*, *Enterobacter cloacae* and *Samonella typhi* attestable to the fact that combination of these plants with chitosan can be an effective therapeutic agent against some of these microorganisms. Also the synergy between *Moringa oleifera* water extracts and Chitosan extracted from prawns was active against *Vibrio cholerae* only. As shown in the studies by Dasola *et al*., (2014) which highlighted how different solvents used in extraction determined the efficacy of the plants properly elucidates the reason why Moringa extracted with water was sensitive to *Vibro cholerae* alone. This shows that water can be a weak solvent in extraction, it is possible that the water used for extraction did not bring out much of the antimicrobial properties needed for this purpose. This study also attests to the fact that solvent's polarities and types have great influence on the various chemical compositions of *M. oleifera* plant extracts due to its various interactions with them (Moyo, 2012).

However, there is an observed contrast with studies carried out by Paul *et al*., (2019) whereby Chitosan, Moringa leaf powder, and their composites at different ratios used in this study inhibited the growth of *Escherichia coli*, *Staphylococcus aureus* with varying diameter. Using a higher ratio of chitosan to lower ratio of moringa leaf powder might produce a better synergistic effect and antimicrobial activity. Also, 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 (Paul *et al*., 2019).

The synergy between *Croton zambesicus* water extracts and Chitosan extracted from Crayfish was effective against *Candida albicans*, *Vibrio cholera*e, *Staphlococcus aureus* and *Salmonella typhi* while the synergy between *Croton zambesicus* water extracts and Chitosan extracted from prawn was effective against *Vibrio cholera*e only which might be as a result of poor solvent extraction. In addition to this, the synergy between *Croton zambesicus* water extracts and Chitosan extracted from Prawns was effective against *Klebsilla oxytoca*, *Enterobacter cloacae* and *Salmonella typhii.* This validated studies carried out by Sabaa *et al*., (2018) and Shih *et al*., (2019).

The *in-vitro* anti-microbial analysis for some extracts showed bacteriocidal and bacteriostatic effect which is also an inhibitory antimicrobial effect as also reported by James *et al*., (2019) thereby confirming the efficacy levels of medicinal plants and its susceptibility (Oguche *et al*., 2019). Although, a plant might exhibit bacteriostatic effect, it might still be effective against microorganism, therefore other factors such as exploring different solvents and other micro-organism before conclusion is important.

In a bid to get the active compounds from the extracts for better comparism, the solvent diethylether was used for extraction as seen in Table 2 above. The solvent extracts (diethylether) of *Croton zambesicus* was susceptible against *Bacillus cetus* and *Eschericha coli* in a wide range of consideration, while the water extracts of *Croton zambesicus* was effective to the same organism at an intermediary range. In correlation with studies carried out by Goy *et al*., (2016) and Tayel *et al*., (2010), the use of more amount or concentrate of extracts in terms of the ratio and solvent have a huge impact on its microbial activities especially for Chitosan. Therefore, it is worth noting that

the potency of the Chitosan extracts may be more accurately evaluated by increasing the concentration, as the zone of inhibition might be influenced by solubility and diffusion rate of the phytocompounds which might be the reason for no reaction with some of the microorganism seen in Tables 1 and 2. These factors taken into consideration will be useful for subsequent research on Chitosan, its commercialization and drug design.

Conclusion

It can be deduced that Chitosan in general can be used as a potential constituent in the quest for developing drugs such as immune boosters against prevailing disease conditions. This deduction is hinged on the fact that Chitosan was sensitive to a wide range of microorganism as shown in this research. Futhermore, the synergism of Chitosan with other ethnomedicinal plants such as *Croton zambesicus*, *Moringa oleifera* and others are potential breakthroughs to new drug design that are cost effective which enhances a wide range of reachability to the community, Nigeria and the world at large.

Recommendation

This research is reproducible and translational. Exploring other solvents and ethnomedicinal plants in synergy with Chitosan for therapeutic purposes shows the need of further research. Furthermore, it is necessary to carry out *in vivo* studies to determine the toxicity of active constituents, their side effects, circulating levels, immunomodulatory effects, pharmacokinetic properties and diffusion in different body sites, for exploitation as an immune booster.

Acknowledgement

We acknowledge the support of the Management of Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria, under the Administration of the Vice Chacellor, Professor Olugbenga E. Ige, for provision of materials and enabling environment for the success of this research. We also thank the Administrative and Technical staff of the Centre for Infectious Diseases Control and Drug Development (CIDCDD), Special Centre, under the University Administration for their encouragement to proceed with our research investigation to obtain desired products.

References

1. Abo K.A., Ogunleye V.O. and Ashidi J.S. (1999). Antimicrobial potential of Spondias mombin, *Croton zambesicus* and Zygotritonia crocea. *Phytotherapy Research*, 13: 494-497.
2. Adjanohoun E.J., Adjakide V. and Desouza S. (1989). Contribution to ethnobotanical and floristic studies in Republic of Benin. Vol.1. Agency for Cultural and Technical Cooperation, p.245
3. Batista, A.C.L., Dantas, G.C., Santos, J. and Amorim, R.V.S. (2011). Antimicrobial Effects of Native Chitosan against Opportunistic Gram-negative Bacteria. *Microbiol J*, 1,105–112.
4. Block S., Stevigny C., Llabres G., deHoffman E., Adjakide V., DePauw-Gillet M. and Quetin-Leclercq J. (2002). Ent-Trachyloban-3β-ol, a new cytotoxic diterpene from *Croton zambesicus*. *Planta Medica*, 68: 647-648
5. Boyle, E.C., Bishop, J.L., Grass, G.A. and Finlay, B.B. (2007). Salmonella: From Pathogenesis to Therapeutics. *Journal of Bacteriological Science*, 189,1489–1495.
6. Boyom F.F., Keumdjio F., Dongmo P.M., Ngadjui B.T., Amvam-Zollo P.H., Menut C. and Bessiere J.M. (2002). Essential oil from *Croton zambesicus* Muell. Arg. growing in Cameroun. *Flavour and Fragrance Journal* 17: 215-217.
7. Clinical and Laboratory Standards Institute (CLSI) (2016). Performance Standards for Antimicrobial Susceptibility Testing. 26th ed. CLSI supplement M100S (ISBN 1-56238- [Print]; ISBN 1-56238-924-6 [Electronic]). Clinical and LaboratoryStandards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087USA, 2016.Google Scholar
8. Dasola A.M., Tunbosun L.A.., Adeyemi A.L. and Abidemi O.O. (2014). Effect of Solvent type on the yields and mineral compositions of leaf extracts of *Moringa oleifera*. *African Journal or Pure and Applied Chemistry* Vol.8(9), pp 134-146.
9. Ganan, M., Carrascosa, A.V. and Martinez-Rodriguez, A.J. (2009). Antimicrobial Activity of Chitosan against Campylobacter spp. and other Microorganisms and its Mechanism of Action. *Journal of Food Protection*, 72, 1735–1738.
10. Goy, R.C., Britto, D. and Assis, O.B.G., (2009). A Review of the Antimicrobial Activity of chitosan. *Polímeros*, 19, 241–247.
11. Goy, R.C., Morais, S.T.B. and Assis, O.B.G. (2016). Evaluation of the Antimicrobial Activity of Chitosan and its Quaternized Derivative on E. coli and S. aureus Growth. *Revista Brasileira de Farmacognosia* 26 (1), 122-127

12. Gupta, A., Gautam M.K., Singh R.K., Kumar M.V., Rao C.V., Goel R.K., Anupurba S. (2010). Immunomodulatory effect of *M. oleifera* extract on Cyclophosphamide induced toxicity in mice. *Indian Jounal of Experimental Biology*. 48. 1157-1160.
13. Hafdani, F.N. and Sadeghinia, N. (2011). A Review on Application of Chitosan as a Natural Antimicrobial. *International Journal of Medical, Health, Biomedical, Bioengineering, and Pharmaceutical Engineering*, 5, (2), 46-50.
14. Islam, M.D.M., Masum, S.M.D. and Mahbub, K.R. (2011). In-vitro Antibacterial Activity of Shrimp Chitosan Against Salmonella para-typhi and *Staphylococcus aureus*. *Journal of the Bangladesh Chemical Society*, 24,185–190.
15. Jia, Z., Shen, D. and Xu, W. (2001). Synthesis and Antibacterial Activities of Quaternary Ammonium salt of Chitosan. *Carbohydrates Research*. 333, 1–6.
16. Koruthu, D.P., N.K. Manivarnan, A. Gopinath, A. Abraham (2011). Antibacterial evaluation, reducing power assay and phytochemical screening of M. oleifera leaf extract: effect of solvent polarity. *International Journal of Pharmaceutical Sciences and Research*. 2 (11). 2991-2995.
17. Kumar, A., and Schweizer, H. (2005). Bacterial resistance to antibiotics: Active efflux and reduced uptake. Advanced Drug Delivery Reviews, 57(10), 1486–1513. https://doi.org/10.1016/j.addr.2005.04.004
18. Maulin, S; Heet, C; Gaurav, D; Rushabh, P; Anjali, B; Sandeep, R (2017) Synthesis and Antimicrobial Properties of Chitosan
19. McNulty, C.A., Boyle, P., Nichols, T., Clappison, P. and Davey, P. (2007). The Public's Attitudes to and compliance with Antibiotics. *Journal of Antimicrobial Chemotherapy*, 60(Suppl 1): i63–i68
20. Moyo, B., P.J. Masika, V. Muchenje (2012). Antimicrobial activities of Moringa oleifera Lam leaf extracts. *African Journal of Biotechnology*. 11(11). 2797-2802.
21. Mwitari, P. G., Ayeka, P. A., Ondicho, J., Matu, E. N., and Bii, C. C. (2013). Antimicrobial activity and probable mechanisms of action of medicinal plants of Kenya: Withania somnifera, Warbugia ugandensis, Prunus africana and Plectrunthus barbatus. *PLoS ONE,* 8(6), 4–12. https://doi.org/10.1371/journal.pone.0065619
22. Ngadjui B.T., Folefoc G.G., Keumedjio F., Dango E., Sondegam B.L. and Conolly J.D. (1999). Crotondiol, a lablane diterpenoid from the stem bark of *Croton zambesicus. Phytochemistry*, 51: 171-174.
23. Oguche J.D., Paul M.S., Ujullu K.E.,Tsaku J.E. and Christ G.G. (2019). Antimicrobial Potential of Chitosan and Moringa oleifera Leaf Powder. *Nigerian Research Journal of Chemical Sciences*. Vol. 7, (ISSN: 2682-6054)
24. Paul, E.D., Garba, Z.N. and James, D.O. (2019). Synergistic-Antagonistic Antibacterial Potential of Chitosan Composites with Moringa oleifera Leaf Powder, *Journal of Applied Sciences and Environmental Management* (JASEM), 23 (4), 759-762
25. Phaechamud, T. (2008). Hydrophobically Modified Chitosans and their Pharmaceutical Applications, *Journal of Pharmaceutical Science and Technology*, 1, 2–9.
26. Rabea, E.I., Badawy, ME-T., Stevens, C.V., Smagghe, G. and Steurbaut, W. (2003). Chitosan as Antimicrobial Agent: Applications and Mode of Action. *Biomacromolecules*, 4, 1457–1465.
27. Sabaa, M. W., Elzanaty, A. M., Abdel-Gawad, O. F., &Arafa, E. G. (2018). Synthesis, characterization and antimicrobial activity of Schiff bases modified chitosan-graftpoly (acrylonitrile). *International Journal of Biological Macromolecules*, 109, 1280-1291.
28. Sharififar, F., S. Pournourmohammadi, M. Arabnejad (2009). Immunomodulatory activity of aqueous extract of Achilleawilhemsii C. Koch in mice. *Indian Jounal of Experimental Biology*. 47. 668-671.
29. Shih P-Y., Liao Y-T., Tseng Y-K., Deng F-S. and Lin C-H. (2019). A Potential Antifungal Effect of Chitosan against *Candida albicans* is mediated via the Inhibition of SAGA Complex Component Expression and the Subsequent Alteration of Cell Surface Integrity. *Front Microbiol* 10: 602. Doi. 10.3389/frmicb.201900602
30. Tayel, A.A., Moussa, S., El-Tras, W.F. Knittel, D. Opwis, K. and Schollmeyer, E. (2010). Anticandidal Action of Fungal Chitosan against *Candida albicans*, *International Journal of Biological Macromolecules*, 47 (4), 454–457.
31. Thilza, I.B., Sanni S., IsahZ.A., SanniF.S., TalleM., and Joseph M.B. (2010). *In vitro* antimicrobial activity of water extract of M. oleifera leaf stalk on bacteria normally implicated in eye diseases. Academia arena. 2(6).
32. Tripathi, S., Mehrotra, G.K. and Dutta, P.K. (2008). Chitosan-Based Antimicrobial Films for Food Packaging Applications. e-Polymers, 93, 1–7.
33. Ugboko H.U., Nwinyi O.C., Oranusi S.U., Fatoki T.H., Omonhinmin C.A. (2020). Antimicrobial importance of medicinal plants in Nigeria, *The Scientific World Journal*. https://doi.org/10.11552020/7059323. Accessed on 24th August, 2021
34. Wagner, H. (1999). Immunomodulator agents from plants. *Institut fur Pharmazeutische Biologie*. Munchen.