# BIOLOGICAL AND CHEMICAL EXTRACTION OF CHITIN AND CHITOSAN FROM THE BLACK SOLDIER FLY (*Hermetia illucens*) EXOSKELETON AND ANTIMICROBIAL ACTIVITY AGAINST SELECTED HUMAN PATHOGENIC MICROBES

**MEVIN KIPROTICH LAGAT**

**MASTER OF SCIENCE**

**(Microbiology)**

**JOMO KENYATTA UNIVERSITY**

**OF**

**AGRICULTURE AND TECHNOLOGY**

**2022**

# Biological and Chemical Extraction of Chitin and Chitosan from The Black Soldier Fly (*Hermetia illucens*) Exoskeleton and Antimicrobial Activity against Selected Human Pathogenic Microbes

**Mevin Kiprotich Lagat**

**A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science in Microbiology of the Jomo Kenyatta University of Agriculture and Technology**

**2022**

## DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Signature…… Date……

**Kiprotich Mevin Lagat**

This thesis has been submitted for examination with our approval as university supervisors.

Signature…… Date……

**Dr. Samuel Were, PhD**

**JKUAT, Kenya**

Signature…… Date……

**Dr. Francis Ndwigah, PhD**

**JKUAT, Kenya**

Signature…… Date……

**Dr Carolyne Kipkoech, PhD**

**Federal Institute of Risk Assessment, Germany**

## DEDICATION

I dedicate this work to my entire family comprising of my mother, Mrs. Naomi Lagat, my brother Mr. Samson Koech, my sister Hellen Lagat and Getrude Rop for giving me humble time during the research period.

**ACKNOWLEDGEMENTS**

I would like to extend my sincere gratitude and thanks to the God Almighty for giving me the patience and strength to endure all the difficulties and distress encountered through the process of completing this work.

Dr. Samuel Were, Dr. Francis Ndwigah and Dr. Carolyne Kipkoech I would like to thank you for the guidance, support and dedication you have shown towards this project.

I would also like to acknowledge the support of the lab technicians at the university for their guidance, positive criticism, and for allowing me to grow as a researcher.

I believe that their wealth of experience and knowledge has steered me on the path to achieving even greater heights in my career.

**TABLE OF CONTENTS**

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF APPENDICES

## LIST OF PLATES

## LIST OF ABBREVIATIONS AND ACRONYMS

**ABSF** Adult Black Soldier Fly

**BSF** Black Soldier Fly

**BSFP** Black Soldier Fly Pupae

**DM** Demineralization

**DP** Deproteination

**FTIR** Fourier-Transform Infrared Spectroscopy

**LAB** Lactic acid bacteria

**MRS** Man Rogosa Sharpe

**SEM** Scanning Electron Microscope

**SPP** Species

**RNA** Ribonucleic Acid

**DNA** Deoxyribonucleic Acid

## ABSTRACT

Globally, the broad-spectrum antimicrobial activity of chitin and chitosan has been widely documented. However, very little research attention has focused on chitin and chitosan extracted from black soldier fly pupal exoskeleton, which are abundantly present as by products from insect-farming enterprises. This study presents the first comparative analysis of chemical and biological extraction of chitin and chitosan from the BSF pupal exoskeleton. The antibacterial activity of chitosan was also evaluated. Traditionally, chitin and chitosan are extracted using chemicals that are both expensive and harmful to the environment. For chemical extraction, demineralization and deproteinization were carried out using 1 M hydrochloric acid at 100°C for 2 h and 1 M NaOH for 4 h at 100°C, respectively. Biological extraction of chitin was carried out by protease-producing bacteria and lactic-acid-producing bacteria for protein and mineral removal, respectively. The extracted chitin was converted to chitosan via deacetylation using 40% NaOH for 8 h at 100°C. Chitin characterization was done using FTIR spectroscopy, while the antimicrobial properties of BSF chitosan were determined using the disc diffusion method. Chemical and biological extraction gave a chitin yield of 10.18% and 11.85%, respectively. A maximum chitosan yield of 6.58% was achieved via chemical treatment. From the FTIR results, biologically and chemically extracted chitin showed characteristic chitin peaks at 1650 and 1550 $cm^{-1}$ wavenumbers corresponding to amide I stretching and amide II bending, respectively. Scanning electron microscopy revealed that the surface morphologies of biologically extracted chitins consisted of fibers and pores together, while chemically extracted chitins had neither fibers nor pores. There was significant growth inhibition for *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* when subjected to 2.5 and 5 g/ml concentrations of chitosan. This findings demonstrate that chitosan from BSF pupal exoskeleton could be a promising and novel therapeutic agent for drug development against resistant strains of bacteria.

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background information

Chitin is a hard, inelastic, and structural polysaccharide found in the exoskeletons of insects, shrimp, lobsters, algae, and fungi (Johney *et al*., 2017). It is the second most abundant polysaccharide after cellulose (Khayrova *et al*., 2020). It arises in nature as ordered micro-fibrils having three polymorphic forms, namely; ∝-chitin, β-chitin, and γ-chitin, respectively (Younes & Rinaudo, 2015).Although being the second most occurring, chitin has been exploited commercially only from marine sources, which has been faced with numerous challenges such as climate change and the seasonality of raw materials (Philibert *et al*., 2017). Previous studies have mainly focused on shrimp, crab, and lobster as the sources of chitin (Hahn *et al*., 2020). The use of insects as an alternative chitin source has grown in popularity over time. This is because they possess enormous biodiversity and have lower levels of inorganic materials, which makes demineralization treatment more efficient (Kaya & Karaarslan, 2015). Although chitin forms the exoskeleton of most insects, it has only been isolated from a few, as shown by studies conducted on grasshoppers, European hornets, and potato beetles (Erdogan & Kaya, 2016; Kaya *et al*., 2014; Kaya & Karaarslan, 2015; Zimri, 2018). The extraction of chitin has not been exhaustively investigated in insects. Recently, most scientists have explored other sources of chitin. For instance, the super worms and green shield bugs could be potential chitin sources (Kaya Asan-Ozusaglam, *et al*., 2017; Oonincx & Finke, 2021). Chitin and chitosan are attracting great attention because of their useful agrochemical and biological properties, which include biodegradability, biocompatibility, non-antigenicity, non-toxicity, and adsorption (Islam *et al*., 2017). These properties have been key in their applicability in the areas of agriculture, medicine, biotechnology, and cosmetics (Yeul & Rayalu, 2013).

This has triggered its extraction on a commercial scale, employing the use of strong chemicals such as strong acids and alkalis, which are faster and more efficient in the production of high yields within a very short time. However, the use of these chemicals in the extraction of chitin and chitosan has a detrimental effect on the environment and is expensive due to this drawback, biological methods of extraction such as the use of plant extracts, enzymes, and the use of bacteria have been developed, which are cheap and eco-friendly (Hajji *et al*., 2014). Chitin from shrimp has been effectively extracted using bacteria such as *Lactobacillus* spp, *Pseudomonas aeruginosa*, and *Bacillus subtilis* (Paul *et al*., 2015). Chitin extracted from shrimp shells using *B. subtilis* fermentation had similar physio-chemical properties to commercial chitin (Philibert *et al*., 2017). A study was conducted on chitin extraction from a fungus (*Phyllophora ribis*) using the following conditions: demineralization (DM) of 2 M Hydrochloric acid (HCl), 60℃, 15 h and deproteination (DP) of 2 M Sodium hydroxide (NaOH), 85℃, 24 h (Erdogan *et al*., 2017). A similar study was conducted using grasshopper and DM conditions of 2 M HCl, 55℃, 1 h and DP conditions of 2 M NaOH, 50℃, 18 h (Kaya Asan-Ozusaglam, *et al*., 2017). However, there are no process conditions that exist for chitin and chitosan extraction from pupae shells and adult black soldier flies or the yields that have been reported. Black soldier fly(*Hermetia illucens*)is a well-known fly that inhabits various countries and does not cause any disease in man and is not found within human settlements (Benelli *et al*., 2014).

Black soldier larvae are dynamic in enhancing sustainability due to their capability to ravenously break down biological waste in an eco-friendly way (Wang & Shelomi, 2017). During mass rearing, large quantities of pupae shells were generated (Zimri, 2018). This motivated the extraction of chitin from the waste materials, which was then used as a source of chitin and chitosan. Black soldier fly inhabits dead bodies during decomposition-initiated oviposition 20-30 days post-mortem, the chitin and chitosan from the black soldier fly have been used for forensic purposes in South America (Ndueze *et al*., 2013).

In Europe, chitosan fibres extracted from BSFL have been used as wound dressers since they possess unique properties such as homeostasis, healing, aesthetic effect, and antibacterial activity, that provide better exudate management (Choi *et al*., 2012).

In China, black soldier fly larvae feed on organic wastes from the kitchen, which are rich in lipids that can be extracted and processed for the production of high-quality biodiesel (Barragan-Fonseca *et al*., 2017). South Africa's Agri-protein limited company produces animal feeds of high protein content and rears black soldier fly larvae that feed on organic waste that would otherwise go into landfills as a waste management scheme that bio-converts organic waste. Various insects have antimicrobial properties that benefit their bodies by protecting them against microbial infection (Barnes *et al*., 2010). A study conducted on the antimicrobial activity of chitin extracted from Blowfly (*Lucilia sericata*) was found to be active against *Staphylococcus aureus*, while *Pseudomonas aeruginosa* was not affected (Barnes *et al*., 2010). A similar study on the antimicrobial activity of black soldier fly chitosan found the following bacteria susceptible: *Klebsiella pneumoniae*, *Neisseria gonorrhoea*, and *Shigella sonnei*. However, chitosan was not active against *Bacillus subtilis*, *Streptococcus mutans*, and *Sarcina lutea* (Choi *et al*., 2012). Studies on the extraction of chitin from black soldier fly exoskeletons have not yet been conducted in Kenya since its rearing is a new technology and its antimicrobial properties have not been evaluated.

### 1.2 Statement of the problem

Antibiotic resistance is one of the biggest threats to global health, food security, and development today. It occurs naturally, but misuse of antibiotics in humans and animals is accelerating the process. A growing number of infections such as pneumonia, tuberculosis, and salmonellosis are becoming harder to treat as the antibiotics used to treat them become less effective, leading to higher medical costs and an increased mortality rate (Blair *et al*., 2015).

While there are some new antibiotics in development, dangerous pathogens such as *Staphylococcus aureus*, *B.sublilis*, *P.aeruginosa* and *Candida albicans* have developed resistance to antibiotics such as methicillin, ampicillin, cotrimoxazole ,streptomycin and sulphathiazole (Zhang *et al*., 2014). When infections can no longer be treated by first-line antibiotics, more expensive medication ought to be used. A longer duration of illness and treatment, often in hospitals, increases health care costs as well as the economic burden on families and societies.

Thus, there is a need for extensive research to seek alternative solutions, which may include the use of herbs. Studies have shown that shrimp-derived chitosan has antimicrobial properties (Mehra *et al*., 2014). The insect rearing is on the rise, with the black soldier fly (BSF) showing enormous success in waste management and the animal feed industry (Barragan-Fonseca *et al*., 2017). Insects have been shown to contain 1-36% of chitin depending on the species. Chitin extraction from insects has previously used strong acids and alkalis, which are detrimental to the environment (Younes & Rinaudo, 2015).The antimicrobial properties of chitosan derived from insects have not been well documented. The study investigated the biological and chemical extraction of chitin and chitosan from the black soldier fly (*Hermetia illucens*) exoskeleton, and its antimicrobial activities against selected human pathogenic bacteria.

### 1.3 Justifications

Black soldier fly farming is becoming more popular, and thus more generation of exoskeleton waste which is a good source of chitin. FTIR characterization of the extracted product will give an insight into whether the biologically extracted chitin was similar to the chemically extracted chitin for validation of the biological extraction method. Biological extraction of chitin and chitosan had the potential to reduce environmental degradation since it decreased the use of strong acids and alkalis in its extraction processes. Antimicrobial resistance is a drawback in the management of diseases. Therefore, there was a need for effective products to effectively manage pathogens that have antimicrobial resistance.

The antimicrobial properties of insect chitin provide new avenues to curb antimicrobial resistance, thereby reducing the disease burden.

## 1.4 Objectives

### 1.4.1 General objective

To characterize and evaluate the chitin yield extracted from the BSF exoskeleton using biological and chemical methods and to determine its antimicrobial activity against human pathogenic microbes.

### 1.4.2 Specific objectives

The specific objectives of the study were

i. To evaluate the yield of chitin extracted from the black soldier fly exoskeleton using biological and chemical methods.
ii. To characterize the structure of BSF chitin and chitosan extracted using different methods.
iii. To investigate the antimicrobial activities of BSF chitosan against selected human pathogenic microbes.

## 1.5 Hypotheses

i. There was no difference between chitin extracted from the BSF exoskeleton by biological and chemical methods.
ii. Chitosan from the BSF had no antimicrobial properties.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Black soldier fly

Black soldier fly (*Hermetia illucens)* is gaining prominence as an insect because of its larva's incredible capacity to consume organic waste fast and convert it into its body mass, which may be utilized as a starting material for economically appealing products (Leni *et al*., 2020). It is among the most important insects farmed in the world (Vogel *et al*., 2018). BSF is a member of the *Stratiomyidae* family and has four distinct life cycles stages: egg, larva, pupa, and imago (Liu *et al*., 2017). This insect feeds only during its larval life and stores enough protein and fat to help it survive on this stored fat and protein during its pupa and imago stage. The adult black soldier fly have a sucking part that enables water drinking, but lack the chewing part in their mouths and depend on proteins and fats stored during the larval stage for their survival (Leni *et al*., 2020). Research has focused on employing BSF larva as an agent for rapid and effective garbage treatment, particularly in poor and middle-income nations (Zimri, 2018), and creating innovative waste treatment systems in which the utilization of BSF larva may aid in the garbage conversion into commercial end products (Abd El- Hack *et al*., 2020).

## 2.2 Structural properties of chitin and chitosan

Chitin is a linear, uncharged polysaccharide polymer made of numerous units of N-acetyl-β-D-glucosamine linked by β-1,4 glycosidic bonds, but some 2-amino-2-deoxy glucopyranose units can occur depending on the biomass and on the procedures used to extract chitin from it (Verma *et al*., 2020).

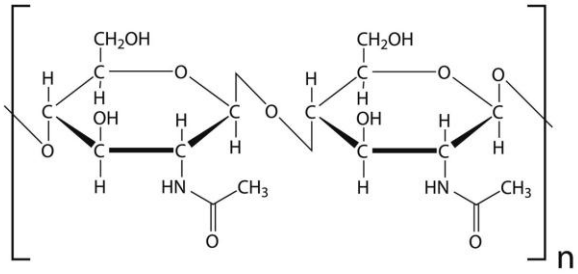

**Figure 2.1: Chemical structure of chitin (Younes & Rinaudo, 2015)**

Structurally, chitin is comparable to cellulose, but for the substitution of the 2-hydroxyl group with an acetyl amino group on each monomer (Kurita, 2020). The presence of the acetyl amino group allows for increased hydrogen bonding between adjacent chitin chains, thereby increasing the strength of the fibrillary scaffold, which is made up of multiple chitin chains (Silva *et al*., 2020). Several chitin chains combine in divergent ways to form crystalline micro fibrils when the chitin polymer is synthesised, forming hydrogen bonds between the N-H and C=O groups as well as between adjacent chains (Lu *et al*., 2013). Chitin is a polymer that is semi-crystalline in structure and, depending on the organism in which it occurs and, on its function, the chitin chains adopt different allomorphs in the solid-state, namely α-, β-, and γ chitin (Victor *et al*., 2020). Alpha-chitin, the more stable and widespread allomorph, occurs where rigidity and mechanical resistance are required, such as in the shells of crustaceans and insects, and it adopts a dense packing due to numerous inter-, intra-chains and interlamellar hydrogen bonds, strongly favoured by the anti-parallel orientation of polymeric chains in the ordered domains (Philibert *et al*., 2017). In β-chitin, which occurs where flexibility and toughness are important, such as in squid pens, the polymer chains are disposed of in a parallel orientation, disfavouring the establishment of hydrogen bonds involving chains pertaining to different lamellae and resulting in a less dense packing compared to alpha-chitin (Daraghmeh *et al*., 2011; Kaya & Karaarslan, 2015; Philibert *et al*., 2017).Gamma chitin, on the contrary, is substantially more uncommon than the other two chitins and some authors claim that it has characteristics of both alpha- and beta-chitin (Erdogan *et al*., 2017). Chitosan, on the other hand, is a linear polysaccharide composed of repeated

units of N-acetyl-2-amino-2-deoxy-2-D-glucopyranose and 2-amino-2-deoxy-2-D-glucopyranose are joined together by - (1–4) - glyosidic linkages. It is known that the amino ($NH_2$) and hydroxyl (OH) groups in their structure are mainly responsible for their properties and potential applications (Mati-Baouche *et al*., 2014).

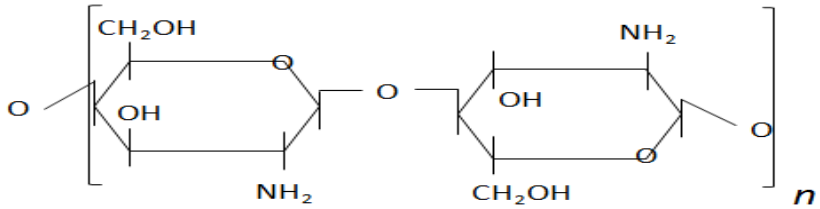


**Figure 2.2: Chemical structure of Chitosan (Younes & Rinaudo, 2015)**

## 2.3 Extraction of chitin

### 2.3.1 Biological extraction of chitin

Extraction of chitin using biological means is emerging as a green, clean, eco-friendly, and economical process. It involves the use of microorganism-mediated fermentation processes, which are highly desirable due to their ease of handling, simplicity, controllability through optimization of process parameters, ambient temperature, and negligible solvent consumption, thus reducing environmental impact and costs (Pachapur *et al*., 2016). The biological methods used for chitin extraction include the use of proteolytic enzymes to digest proteins and a fermentation process using microorganisms that allow protein and mineral digestion (Philibert *et al*., 2017). Alcalase, pepsin, papain, and trypsin are proteolytic enzymes that digest proteins. They also reduce depolymerization in chitin extraction (Younes & Rinaudo, 2015). Commercially purified protease enzymes can be used but they are expensive. Hence, crude protease enzymes are mostly preferred as they can be accessed and is cheaper. Extraction using bacteria can be done by two major groups, namely, lactic acid-producing and non-lactic acid-producing bacteria. Lactic acid fermenting bacteria include; *Lactobacillus plantarum*, *Lactobacillus piracies*, *Lactobacillus fermentum* and *Serratia marcescens* (Philibert *et al*., 2017).

Lactic acid bacteria react with the calcium carbonate constituent of the black soldier fly exoskeleton, which results in the formation of calcium lactate that will be precipitated and detached during the demineralization process (Philibert *et al*., 2017). The effectiveness of lactic acid fermentation depends on, species, quality of inoculum, carbon sources, concentration, initial pH, pH after the fermentation process, and temperature (Younes & Rinaudo, 2015). *Pseudomonas* spp., *Bacillus* spp. and *Aspergillus* spp. are examples of non-lactic producing microorganisms used in chitin extraction (Casadidio *et al*., 2019).

### 2.3.2 Chemical extraction of chitin

This is the traditional chitin extraction method, which consists of three steps: demineralization, deproteinization, and decolourization (Kaya, Erdogan, *et al*., 2015). During demineralization, the material is treated with hydrochloric acid to remove minerals such as calcium carbonate and calcium phosphate to form soluble calcium salts, and carbon dioxide is released in the process (Moussout *et al*., 2016). Excess salts are then filtered to separate the solid phase and liquid phase; the solid phase is then rinsed with deionized water to remove excess acid. This is followed by treatment with NaOH for deproteinization, followed by rinsing with distilled water (Kaur & Dhillon, 2015). The last step involves the decolourization process, which involves removing pigments and lipids by using organic solvents such as acetone (Tourón *et al*., 2016).

## 2.4 Chitin sources and yield conditions

Several studies have been conducted by various researchers on chitin extraction for various applications such as, in agriculture, medicine and food industry. They used various variables such as concentration of the reactants, temperature, and reaction time, which have resulted in variation in the yield produced.

In a study on the chitin extraction from woodlouse (*Oniscus oscellus*), DP (Deproteination) conditions used were 4 M NaOH, 150℃, 18 h, and DM (Demineralisation) conditions used were 4 M HCL, 75℃, 2 h, which produced a 6.5% chitin yield (Kaya & Karaarslan, 2015).

A similar study using *Leptinotarsa decemlineata* (potato beetle) with the DM conditions of 2 M HCL, 75℃, 2 h and DP conditions of 2 M NaOH, 50℃, 18 h produced a yield that ranged from 18 to 21%, which was slightly higher than that of 6.5% because of the decrease in HCL and NaOH concentrations as well as the reduction in temperature (Kaya & Karaarslan, 2015).

In a study conducted on the extraction of chitin from the tilapia scale, DP conditions used were 0.25 M NaOH, 50℃, 3 h and DM conditions of 0.5 M HCL, 25℃, 2 h, which produced a higher yield of 20% chitin (Boarin-Alcalde & Graciano-Fonseca, 2016). At room temperature, extraction of chitin from crickets using 4 M NaOH, 98℃ for 2 h and 1 M HCL yielded 40% (Kipkoech *et al*., 2017). A study was conducted on *Palomena prasina* and the DP conditions used were 2 M NaOH, 140℃, 20 h with DM conditions of 2 M HCL,100℃, 2 h, which produced a chitin yield of about 10.8% (Kaya-Asan-Ozusaglam, *et al*., 2017).

Chitin extraction from grasshoppers (*Dociostaurus maroccanus*) used DP conditions of 2 M NaOH, 50℃, 18 h, and DM conditions of 2 M HCL, 55℃, 1 h, which increased yield production by 13% (Kaya *et al*., 2016). A study on super worms used DM conditions of 1 M HCL, 35℃, 30 min and DP conditions of 0.5 M NaOH, 50℃, 18 h, leading to a decrease in yield production of 5% (Oonincx & Finke, 2021). Extraction of chitin from fungus (*Lactarius vellereus*) used DM conditions of 2 M HCl, 60℃, 15 h and DP conditions of 2 M NaOH, 85℃, 24 h, which then resulted in a yield of 11.4% (Kaya *et al*., 2016). On the contrary, chitin extraction from *Hermetia illucens* was done using DM conditions of 1 M HCL at 50℃, for 100 mins and DP conditions of 1 M NaOH, 85℃, 10 h, which then resulted in a dry weight of 13% chitin from black soldier fly pupae (BSFP) shells and a 5% chitin yield from adult black soldier flies (Zimri, 2018).

**2.5 Chitin deacetylation**

Deacetylation serves to remove acetyl groups from chitin, resulting in chitosan, which affects the physicochemical and biological properties, thus determining its application suitability. It can be done either through a chemical or enzymatic method (Yeul & Rayalu, 2013).

### 2.5.1 Chemical deacetylation method

After the chitin extraction process, the mixture is mixed with a concentrated sodium hydroxide solution to produce chitosan that has a different degree of deacetylation, which is dependent on the temperature, reaction time, and concentration of the sodium hydroxide used (Ahing & Wid, 2016). The conditions favourable for the optimal conversion of chitin to chitosan are, a 40-60% concentration of sodium hydroxide, a temperature of 80 to 150℃ and a time interval between 2 to 10 h respectively (Kaya & Karaarslan, 2015). Sodium hydroxide hydrolyses the acetyl groups, converting the N-Acetyl-D-glucosamine units to D-glucosamine units (Philibert *et al*., 2017).

### 2.5.2 Enzymatic method

Chitin can be deacetylated by the use of the chitin deacetylase enzyme, which is derived from various sources such as bacteria, fungi, and even insects. Bacteria such as *Bacillus* and *Serratia* species have been known to produce the chitin deacetylase enzyme (Zhou *et al*., 2010). Various fungi have also been used to produce the enzyme chitin deacetylase, such as *Aspergillus nidulans* and *Mucor rouxii.* In fungi, these enzymes are glycoproteins secreted in the periplasm or even in the culture medium. Chitin deacetylase extract from a fungus, *Mucor rouxii,* catalyses the conversion of chitin to chitosan by deacetylation of N-acetyl-D-glucosamine residues (Zhou *et al*., 2013).

## 2.6 Applications of chitin and chitosan

The interest and demand for chitin over the years have increased and are rapidly growing as potential new applications are virtually discovered every day (Usman *et al*., 2016).

It is because of their biological attributes such as biodegradability, biocompatibility, non-antigenicity, and non-toxicity that make them useful biopolymers which have seen them find applications in various sectors such as agriculture, pharmaceuticals, medicine, and water treatment (Nechita *et al*., 2017).

Chitosan polymer has been used as an emulsifying, antimicrobial ,thickening and stabilizing agent in food manufacturing (Joseph *et al*., 2021). They have shown enormous bioactivity in the medical field.

For example, in wound healing, chitosan increases dermal regeneration, which accelerates wound healing by preventing bacterial infiltration and preventing water loss (Xia *et al*., 2020). Chitosan is also used as a surgical thread since they are strong, flexible and decompose after the heals (Zheng *et al*., 2021). Chitosan polymers have been used in orthopaedic applications; chitin-based materials are suitable candidates for collagen replacement (Islam *et al*., 2017). Chitin hydroxyapatite composite is mechanically flexible, which enhances bone formation and impermanent artificial ligaments for the knee joint (Li *et al*., 2021).

Chitin and chitosan have been utilized in drug delivery in the form of hydrogels, which are highly swollen, hydrophilic polymer networks that can absorb enormous amounts of water. Site-specific medication administration to the gastrointestinal tract might be possible using hydrogels (Spiridon *et al*., 2021). Chitin and chitosan have been applied as tablets and are reported to be useful diluents in pharmaceutical preparations (Barragan-Fonseca *et al*., 2017).

In biotechnology, chitin and chitosan have been utilised for enzyme mobilization since chitosan-based materials are suitable enzyme mobilisers since they are specific, efficient, operate under mild conditions, are unstable, after isolation and purification, are biocompatible, biodegradable, have reactive functional groups, and have high affinity for proteins (Verma *et al*., 2020). Also, chitin and chitosan-based biomaterials have been used in gene delivery as they have high transfection efficiency (Gao *et al*., 2008).

Chitosan polymers have been utilised in water treatment for the removal of metal ions and dyes (Nechita, 2017).

They have also been applied in pulp and paper surface treatment on photographic papers (Kansal *et al*., 2020). In the cosmetics industry, they have been used in makeup powder and nail polish (Alves *et al*., 2020). In agriculture, chitosan polymer has been used as a seed and leaf coating plant protectant (Maluin & Hussein, 2020). Approximately $10^{11}$ tons of chitin are produced annually worldwide in the marine environment and only 150,000 tons are commercialized (Zaku *et al*., 2011).

## 2.7 Characterization of chitin and chitosan

Biological materials are best utilized after characterization. Currently, chitin and chitosan-based materials have been applied to various fields of science, such as medicine and agriculture, due to their unique properties such as biodegradability, biocompatibility, non-antigenicity, non-toxicity, and adsorption (Kaya & Karaarslan, 2015).

### 2.7.1. Transform Infrared Spectroscopy (FTIR)

FTIR is used to determine the functional groups, differentiate between the two chitin isomorphs and check for impurities in chitin and chitosan preparation to determine the degree of acetylation and deacetylation and to control purification conditions (Kaya, Baran, Asan-Ozusaglam, *et al*., 2017). It uses infrared light to scan test samples and observe chemical properties depending on transitions between vibrational energy states, stretching and bending, of a molecule (Kaya Asan-Ozusaglam, *et al*., 2017). Because of its relative equipment availability, simplicity, and independence from sample solubility, FTIR spectroscopy is one of the most frequently used characterization techniques for chitin and chitosan.

### 2.7.2 Scanning Electron Microscopy (SEM)

This is a microscopic technique that is used for visual confirmation of the morphology and physical state of the surface (Cui *et al*., 2020). A scanning electron microscope can image samples distributed over a surface of any size and shape, and it can offer surface imaging up to a resolution of < 2 nm (Akhtar *et al*., 2018). In addition to the polymeric material, SEM is better for imaging bulk samples and has a greater depth of field, giving rise to better 3D images of the sample (Gholinia *et al*., 2020).

SEM images are capable of resolving the morphology of the fibers as well as detecting fiber diameters (Amanzadeh *et al.*, 2018). SEM remains a quick method for observing the fibers produced, and it requires a very small sample size for its operation (Amanzadeh *et al*., 2018). Transmission electron microscopy is another alternative for detecting fiber diameters with extremely small fibers (Chen *et al*., 2020).

## 2.8 Antimicrobial properties of chitin and chitosan

Innumerable researchers conducted their earlier studies on the antimicrobial activities of various natural products in an attempt to combat the escalating microbial diseases that have become resistant to conventional antibiotics. These natural products used come from various sources, which include beneficial plants, insects, fungi, bacteria, and algae. Chitin is such a natural product that has gained enormous attention for years.

Chitosan from mushroom species has shown remarkable activity against bacterial pathogens such as *S. aureus*, *E. coli*, *B. subtilis*, *P. aeruginosa*, *K. pneumoniae*, and fungal pathogens such as *A. niger* and *A. flavus* (Johney *et al*., 2017). A similar study was conducted on the antimicrobial properties of chitosan extracted from shrimp shell waste against *S.aureus, E. coli, B. subtilis, P. aeruginosa,* and *K. pneumoniae* (Tareq *et al*., 2013).Chitosan from crab shell waste, on the other hand, has been found to exhibit stronger bactericidal activity for gram-positive bacteria than gram-negative ones and was effective against *E. coli, Pseudomonas fluorescens, S. typhi, P. aeruginosa, B. subtilis,* and *S. aureus* (Ahmed *et al*., 2017).

A study conducted on chitosan from housefly (*Musca domestica*)showed it to have antibacterial activities against gram-positive and gram-negative bacteria, which then showed a positive effect against *S. aureus, P. aeruginosa* and did not have any effect against *E. coli* (Choi *et al*., 2012). The antimicrobial activity of black soldier fly larvae chitosan against pathogenic bacteria such as *E. coli*, *Salmonella enterica*, *D-streptococci*, *Coliforms*, and *C. perfringens* has been reported (Gabler, 2014; Spranghers *et al*., 2017). However, the antimicrobial activity of black soldier fly exoskeleton has not been done on *S. aureus*, *C.albicans*, *B. subtilis* and *P. aeruginosa*.

## 2.9 Mechanism of action of chitosan on bacteria

Antimicrobial activities of chitin and chitosan against a wide variety of microorganisms have been widely studied (Islam *et al*., 2017).

Chitin has been shown to have no substantial antimicrobial activity because it is naturally uncharged and insoluble (Ólafsdóttir *et al*., 2020). However, chitosan has been found to have antifungal, antibacterial, and antiviral activities (Mohamed & Madian, 2020). Chitosan also inhibits the growth of many pathogenic bacteria, for example, *E. coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa.* However, its antimicrobial activities are higher against fungi than bacteria, and among bacteria, it is often higher against gram-positive than gram-negative organisms. This could be due to the differences in their surface structures and cell wall composition (Xing *et al*., 2017). Different mechanisms for the direct antibacterial activities of chitosan have been proposed and described. The main mechanism of action proposed is related to its cationic properties (Chandrasekaran *et al*., 2020). The mechanism involves electrostatic interactions where the positive charges on the chitosan molecules interact with the negative charges on the surface of the pathogens (Fuster *et al*., 2020). This interaction leads to the destruction of cell structure, extensive alterations of the cell surface, and increased permeability of the cell membrane. Another proposed mode of action of chitosan involves the alteration of cell permeability (Inbaraj *et al*., 2020). This includes its deposition on the pathogen cell surface, resulting in the creation of an impermeable polymeric layer that inhibits the uptake of nutrients in the cell and changes in metabolite secretion in the extracellular matrix (Xing *et al*., 2017). Chitosan is also capable of chelating some essential nutrients, trace elements, and metals that are necessary for microbial growth, thus inhibiting their growth and toxin production (Xing *et al*., 2017).

### 2.10 Mechanism of action of chitosan on fungal pathogens

In contrast to gram positive and gram-negative bacteria, the difference between chitosan sensitive and -resistant fungi is less pronounced. First, chitosan affects the cell membrane via electrostatic interactions with the negatively charged phospholipids(Ma *et al*., 2017) Once the cell membrane is disrupted, chitosan is capable of entering the cell (Meng *et al*., 2020).

This could lead to inhibition of DNA/RNA synthesis (Divya *et al*., 2017) and disruption of protein synthesis (Li & Zhuang, 2020). However, for chitosan-resistant fungi, it seems that chitosan is unable to permeate the cell membrane and remains at the outer surface (Verlee *et al*., 2017). This is in contrast to chitosan-sensitive fungi, where disruption of the cell membrane is observed together with leakage of intracellular material, and penetration of chitosan into the cell membrane (Riaz Rajoka *et al*., 2020). The reason why chitosan is unable to disturb the cell membrane for chitosan-resistant fungi is due to the difference in fluidity of the cell membrane (Lopez-Moya *et al.*, 2021).

Chitosan proved important antifungal activity, even if the mechanisms of action are less studied that those involved in the antibacterial activity. Based on the available data, fungi appear to be more sensitive to antimicrobial activity of chitosan than bacteria (Riaz Rajoka *et al*., 2020). The antifungal activity of chitosan depends on several factors as molecular weight, deacetylation degree, chitosan concentration and pH (Confederat *et al*., 2021). The influence of the molecular weight and deacetylation degree seems to be dependent on tested strains (Riaz Rajoka *et al*., 2020). Concerning chitosan concentration, values between 1% and 5% proved to ensure an optimal antifungal activity (Confederat *et al.*, 2021). Similar to antibacterial activity, the antifungal activity of chitosan is greater at lower pH values (Perinelli *et al*., 2018).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Study site

This research was conducted at the Jomo Kenyatta University of Agriculture and Technology (JKUAT), 30 km north-east of Nairobi metropolis, at the Government of Kenya (GoK) Botany Laboratory (10180S, 370E). The exoskeleton used to extract chitin and chitosan was obtained from the JKUAT black soldier fly farm located at the main campus in Juja.

## 3.2 Chemical extractions of chitin from the Black soldier fly (*Hermetia illucens*) exoskeleton

The BSF was reared following the standard operating procedures at the JKUAT farm (Shumo *et al*., 2019). Five kilograms of the eighteen days old pupal exuviae samples were collected after three independent rearing periods in April, July, and September 2020. The exoskeletons were sorted, washed in water, dried and blended into a fine powder using a commercial laboratory blender (Sanyo®). The experiment was carried out in hood chamber in the laboratory. One hundred grams (100 g) of the resultant powder was treated with 1000 ml of 1 M sodium hydroxide (analytical grade) and was allowed to boil for 4 h with continuous stirring to remove proteins. The solid product was thoroughly washed with distilled water until a neutral pH was achieved. It was then dried in a hot air oven at a temperature of 60℃ for 24 h. Calcium carbonate was removed from the dried sample by treating it with 1000 ml of 1 M hydrochloric acid (analytical grade) and allowed to boil for 2 h. The final product was rinsed in distilled water until a neutral pH was achieved. It was then dried in a hot air oven at a temperature of 60℃ for 24 h.

The final dried product was weighed and the percentage yield of chitin was calculated from the initial exoskeletons used. All procedures were carried out in triplicate.

### 3.3 Biological extractions of chitin from Black soldier fly (*Hermetia illucens*) exoskeleton

#### 3.3.1 Bacteria isolates

Biological extraction of chitin was done following the method of Aytekin & Elibol, 2010 with slight modifications using protease-producing bacteria (*Bacillus subtilis* and *Pseudomonas aeruginosa)* for deproteination which produced proteolytic enzymes which cleaved protein bonds in the exoskeleton hence effective in deproteination and lactic acid bacteria (*Lactobacillus plantarum*) this bacteria produced lactic acid which reacted with the calcium carbonate component of the exoskeleton to precipitate calcium lactate hence effective for demineralisation. The isolates used were obtained from JKUAT food microbiology and botany laboratories. The isolates were stored at 4℃ freezers until next used. The protease producing bacteria (*Bacillus subtilis* and *Pseudomonas aeruginosa)* were cultured in Nutrient Broth while lactic acid producing bacteria (*Lactobacillus plantarum*) was cultured in Man De Rogosa Sharpe broth. These isolates were used because they have been reported to give high yields in chitin extraction (Pachapur *et al*., 2016).

#### 3.3.2 Deproteinization and demineralization of chitin

In the first set-up, deproteinization was performed first using proteolytic bacteria, namely, *Bacillus subtilis* and *Pseudomonas aeruginosa*, followed by demineralization using *Lactobacillus plantarum*. In the second set of experiments, demineralization was performed first, followed by deproteinization. Each of the sets of experiments was replicated three times to increase the reliability and accuracy of the results obtained, and repeated for the three different independent samples.

During the deproteinization process, 100 g of the exoskeleton flour was soaked in 2 liters of distilled water. To this mix, 55 g of sucrose was added and the mixture was then inoculated with 10 ml of *Bacillus subtilis* and *Pseudomonas aeruginosa* and incubated at 37℃ for 5 days.

The deproteinized material was then sterilized and demineralized by inoculating with 10 ml of *Lactobacillus plantarum* and incubated at 37℃ in anaerobic verts for another 5 days (Kaur & Dhillon, 2015). For co-cultivation of *Bacillus subtilis* and *Pseudomonas aeruginosa* in chitin extraction, 100 g of the exoskeleton flour was soaked in 2 L of distilled water. To this mix, 55g of sucrose was added and the mixture was then inoculated with 10 ml of *Bacillus subtilis* and *Pseudomonas aeruginosa* and incubated at 37℃ for 5 days. After 5 days, the deproteinized material was sterilized and then demineralized by inoculating it with 10 ml of *Lactobacillus plantarum* and incubated at 37℃ in anaerobic verts for another 5 days.

### 3.3.3 Chitin deacetylation to chitosan

The extracted chitin from the Black soldier flies exoskeleton using chemical method was then converted into chitosan by refluxing 200 g in a 40% NaOH (1000 ml) solution and boiling for 8 h. The deacetylated chitosan was then cleaned with sterile water to neutral pH and dried in a hot air oven at 60℃. The dried samples were then stored at 4℃ until used.

### 3.3.4 Determination of percentage yield of chitin and chitosan

The chitin yield was calculated based on the dry weight using gravimetric measurements between the raw pupae shell and the chitin that was obtained after the chitin extraction process. Furthermore, the chitosan yield was calculated based on the dry weight from the weight difference between the chitin that was obtained and the chitosan that was obtained after the chitosan production process using the following equations;

$$\text{Chitin yield (\%)} = \frac{a}{b} \times 100 \quad \ldots\ldots\ldots\ldots 3.1$$

Where **a** was the obtained chitin weight (g) and **b** was the weight of the pupae's shell (g).

$$\text{Chitosan yield (\%)} = \frac{c}{d} \times 100 \ldots\ldots\ldots\ldots\ldots 3.2$$

Where **c** was the weight (g) of chitosan that was obtained, while **d** was the weight (g) of chitin that was prepared.

### 3.4 Characterization of chitin and chitosan by Fourier transform infrared spectroscopy (FT-IR)

Dried chitin and chitosan samples were mixed with potassium bromide (KBr) and ground into a fine powder. The dried mixture was then pressed under a vacuum in a mold to form a KBr disc containing the sample. Fourier transform infrared spectroscopic (Bruker Ever, Belgium) measurements were then performed at a scanning range of 4000 to 400 $cm^{-1}$ (Akram *et al*., 2012).

### 3.6 Scanning Electron Microscope (SEM) analysis

A scanning electron microscopy analysis was performed to determine the differences between the surface morphologies of biologically and chemically extracted chitin and chitosan from BSF pupae shells along with commercial shrimp chitin and chitosan. The surface morphologies of chitosan and chitin samples were determined using a scanning electron microscope (JEOL NeoScope JCM-7000Nova SEM). The samples were coated with carbon film and examined using the secondary electron mode with an accelerating voltage of 15 Kv to show microstructures at different magnifications.

### 3.7 Antimicrobial activities

#### 3.7.1 Test organisms for antimicrobial assay

The antimicrobial activities of chitosan were tested against two Gram-negative bacteria: *Escherichia coli* ATCC®25922 (*E. coli*), *Pseudomonas aeruginosa* ATCC®27853 *(P. aeruginosa*), and two Gram-positive bacteria: *Staphylococcus aureus* ATCC®25923 (*S. aureus*), *Bacillus subtilis* ATCC®11778 *(B.subtilis*), and yeast *Candida albicans* ATCC®10231 (*C. albicans*). The test microbes were obtained from medical microbiology and GoK-Botany laboratories, JKUAT.

### 3.7.2 Inoculum and sample preparation

Each bacterial strain and *C. albicans* were sub-cultured overnight at 37℃ in nutrient broth, and sabouraud dextrose broth and then on their agar plates, respectively. Bacterial colonies were then suspended in 9 ml of sterile saline solution and the suspension was adjusted to achieve turbidity equivalent to the 0.5 McFarland standard, which is equivalent to 1 $x10^8$ colony forming units (CFU)/ml. Mueller Hilton agar was then prepared and 12 ml of the media was dispensed to the Petri-dishes and allowed to solidify. Different concentrations (0.5.1, 2.5, and 5g/ml) of BSF chitosan were prepared by dissolving chitosan in 1% acetic acid solution.

### 3.7.3 Antimicrobial susceptibility assay

The Kirby-Bauer disc diffusion method was employed for antimicrobial assay (Kourmouli *et al*., 2018). Twenty; microlitres (20 µl) of freshly prepared McFarland bacterial culture of *E. coli*, *S. aureus, P. aeruginosa*, *B subtilis*, and fungal culture of *C. albican*s were then inoculated and spread uniformly onto Mueller Hilton agar plates.
Chitosan sample discs, prepared by impregnating 50 µL of chitosan solution onto sterile filter paper discs (6 mm) followed by air-drying, were placed on the top of the agar plates. The plates were then incubated at 37℃ for 24 h. The presence of inhibition zones was measured around each disc in millimetres (mm) and considered evidence of antimicrobial activity. The experiments for each test organism were carried out in triplicates. Filter paper discs soaked in 2 ml of 1% acetic acid without chitosan were used as a positive control and sterile distilled water was used as a negative control. The effects of BSF chitosan were also compared to a group of standard reference antibiotics containing; Ampicillin (25 mcg), Streptomycin (25 mcg), Tetracycline (100 mcg), Nalidixic acid (30 mcg), Chloramphenicol (25 mcg), Clotrimazole (25 mcg), and Nitrofurantoin (200 mcg) to ascertain whether BSF chitosan can be used as an antibiotic compound.

### 3.8 Data collection and analysis

Data on chitin yield was collected based on the dry weight using gravimetric measurements between the raw pupae shell wastes and the chitin that was obtained after the extraction process. Data on the chitosan yield was collected based on the dry weight of the weight difference between the chitin that was obtained and the chitosan that was obtained after the chitosan production process. The data on antimicrobial activity was collected by measuring clear zones of inhibition around each disc on each plate of each test organism. Data analysis was done using Stata SE-64 2011 statistics software and means were separated using the Bonferroni range test. Data on the chitin and chitosan yield was expressed as mean ± standard deviation, and comparisons of mean yield among groups were performed by one-way analysis of variance. Similarly, the difference in mean inhibition zone was done using a one-way analysis of variance, and the difference was considered significant at $p \leq 0.05$.

# CHAPTER FOUR

# RESULTS

## 4.1 Chitin yield from the exoskeleton of the black soldier fly

The chitin yield obtained through co-cultivation of bacteria was not significantly different from the yield obtained from chemical extraction. The chitin yield obtained from co-cultivation of bacteria was higher than chemical extraction, though it was not significant (Table 4.1). There was no significant difference between the chitin yield obtained from the chemical extraction and the yield obtained from the extraction using *P. aeruginosa* and the reverse order of *B. subtilis*. There was, however, a significant difference between the chitin yield obtained from co-cultivation of bacteria and all the chitin yield obtained from individual extraction of chitin. When the reverse order of extraction was employed (demineralisation followed by deproteination), there was an increase in the yield of chitin, with *B. subtilis* giving a higher yield than *P. aeruginosa*.

**Table 4.1: Mean chitin yield (g) from biological and chemical extraction from black soldier pupae exoskeleton**

| Chitin extraction treatments | Order of extraction | Chitin Yield |
|---|---|---|
| (*P. aeruginosa* + *B. subtilis* )+*L. plantarum*(Co-ferm) | Deprot-Demin | $11.85\pm1.16^{a}$ |
| Chemical extraction | Deprot-Demin | $10.18\pm0.42^{ab}$ |
| *P. aeruginosa* + *L. plantarum*(Ind-ferm) | Deprot-Demin | $9.47\pm0.52^{bc}$ |
| *L.plantarum* + *B. subtilis*(Ind-ferm) | Reverse | $8.76\pm0.88^{bc}$ |
| *L.plantarum*+*P.aeruginosa*(Ind-ferm) | Reverse | $7.99\pm1.16^{c}$ |
| *B. subtilis* + *L. plantarum*(Ind-ferm) | Deprot-Demin | $7.78\pm0.68^{c}$ |
| P-value | | < 0.05 |

Different letters in the same column indicate significant differences ($p < 0.05$). Values were given as mean ± standard deviation.*ind-ferm- individual fermentation, *Co-ferm-co-fermentation.

### 4.2 Analysis of functional groups in chitin extracted from the Black Soldier fly

In this study, chemically and biologically extracted chitins were characterized using an FTIR spectrophotometer and compared to standard commercial chitin (Figure 4.1). There were no differences observed in the structure of chitin extracted through biological and chemical methods.

Biologically extracted chitin exhibited vibration peaks at 3440, 2355, 1650, and 1550 $cm^{-1}$ wavenumbers. Chemically extracted chitin showed vibration peaks at 3440, 2355, 1651, and 1552 $cm^{-1}$ wavenumbers. Commercial shrimp chitin had peaks at 3440, 2355, 1652, and 1552 $cm^{-1}$ wavenumbers.

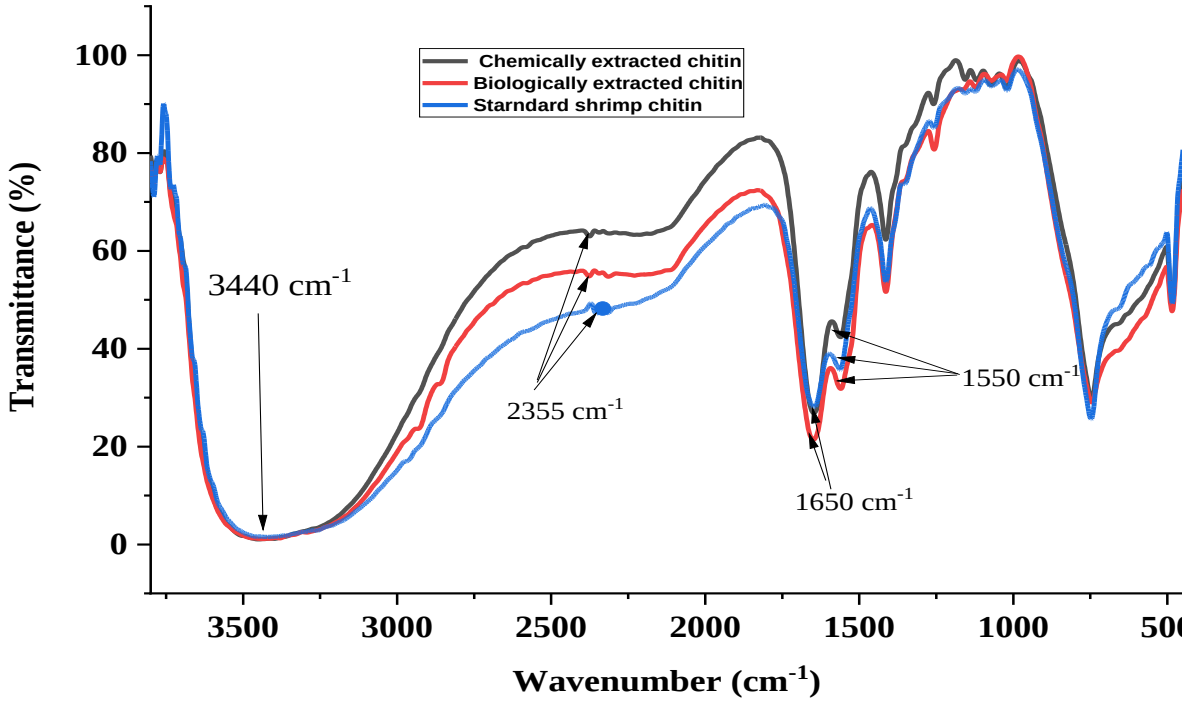


**Figure 4.1: FTIR spectra showing comparison between chitins extracted from BSF pupae shells using biological and chemical method alongside with commercial standard shrimp chitin**

### 4.3 Analysis of functional groups in chitosan extracted from the Black Soldier fly

In this study, chemically extracted and standard chitosan was analysed using FTIR to verify the presence of the characteristic peaks. The FTIR results suggested that there was a similarity between the chemical composition and bonding types of chitosan in the BSF pupae shells and commercial shrimp chitosan (Figure 4.2). The characteristic bands were recorded at 1650 $cm^{-1}$ and 1587 $cm^{-1}$ for the chitosan from BSF, and at 1649 $cm^{-1}$ and 1587 $cm^{-1}$ for commercial chitosan.

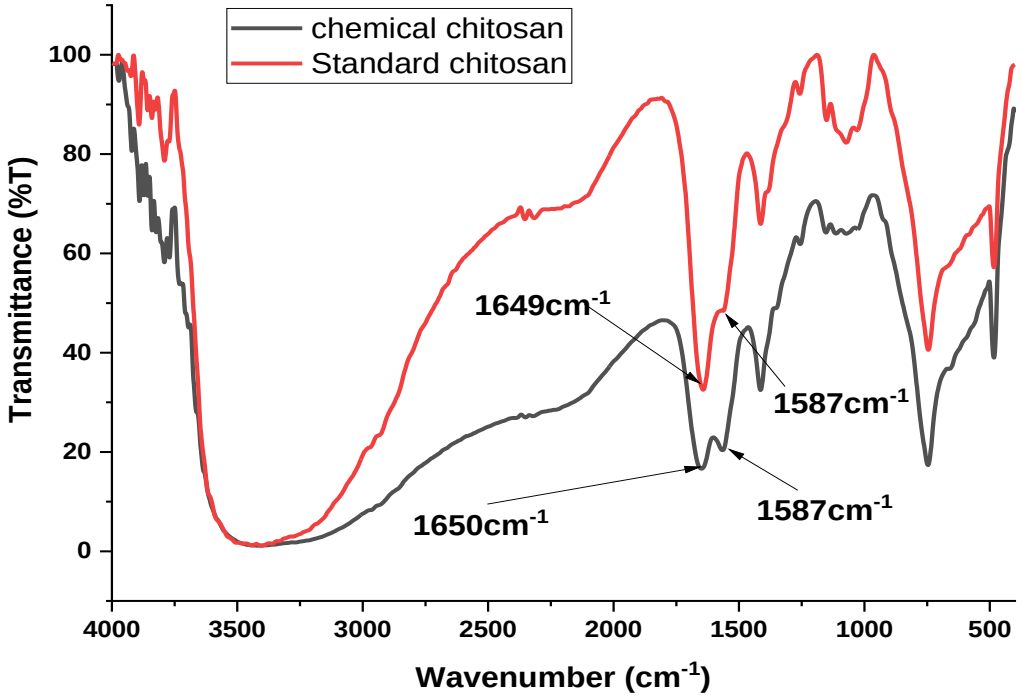


**Figure 4.2: FTIR spectra of chemically extracted BSF pupae exoskeleton chitosan compared to the standard shrimp chitosan**

### 4.4 Scanning electron microscopy analysis of chitin and chitosan from Black soldier fly exoskeleton

The surface morphologies of chitins obtained from BSF pupae exoskeleton through *B. subtilis, P. aeruginosa,* and co-cultivation showed a rough, tightly packed structure with repeating circular and hexagonal units in a honeycomb-like arrangement (Plate 4.1 a, b, and c). The biologically extracted chitins had rough surfaces with fibers having pores. The pore sizes were 50 µm in diameter at X500 magnification.

An increase in magnification X1000 revealed that all the biologically extracted chitins had fibers and pores, but low magnification gave clearer micrographs. Chitin obtained by chemical means had a smooth surface with few fibers and pores, and the surface morphologies were tightly packed structures with repeating circular and hexagonal units in a honeycomb-like arrangement (Plate.4.1d). Commercial shrimp chitin had a rocky smooth surface, without fibers, and numerous pores of 100 um in diameter at X500 magnification (Plate.4.1e).

Biologically and chemically extracted chitin from BSF pupae shells were similar as both chitins had rough surface morphology and same shape, However, biological chitin had fibers and pores together which were absent in chemically extracted chitin. They differed from commercial shrimp chitin in that commercial shrimp had a smooth surface, a rocky-like shape, and numerous pores but no fibers.

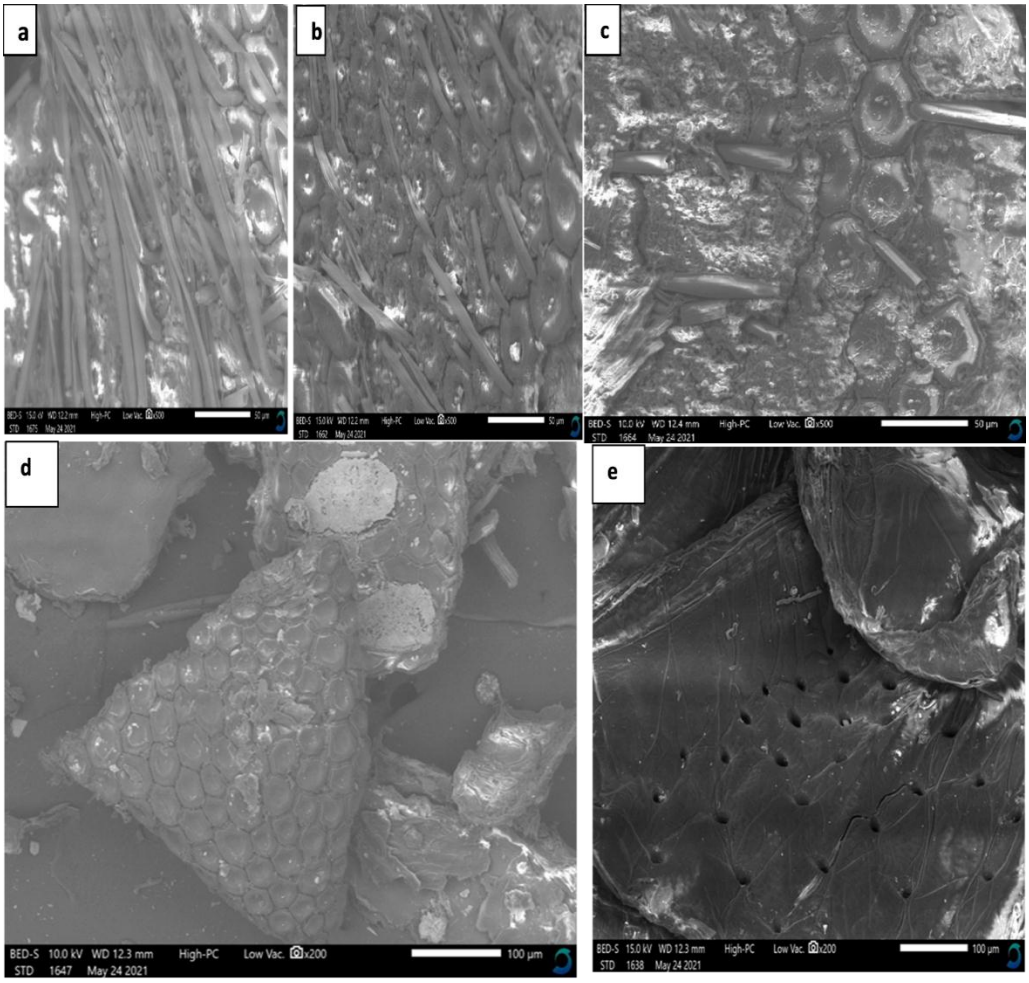


**Plate 4.1: Scanning electron micrographs of a) Chitin from BSF pupae shells extracted through *B. subtilis* bacteria, b) Chitin from BSF pupae shells extracted through *P. aeruginosa* bacteria, c) Chitin from BSF pupae shells extracted through co-cultivation of *B. subtilis* and *P. aeruginosa* bacteria, d) Chitin from BSF pupae shells extracted by use of chemicals, and e) Commercial shrimp chitin.**

BSF pupae exoskeleton chitosan obtained through chemical means had a rough surface, without any fibers and/or pores on both surfaces (Plate. 4.2 a), while commercial shrimp chitosan had a dense, smooth surface without fibers and pores (Plate. 4.2 b).

BSF pupae exoskeleton chitosan obtained through chemical means had a rough surface, without any fibers and/or pores on both surfaces (Plate.4.2 a), while commercial shrimp chitosan had a dense, smooth surface without fibers and pores (Plate.4.2 b). Commercial shrimp chitosan had a dense, smooth surface without fibers and/or pores.

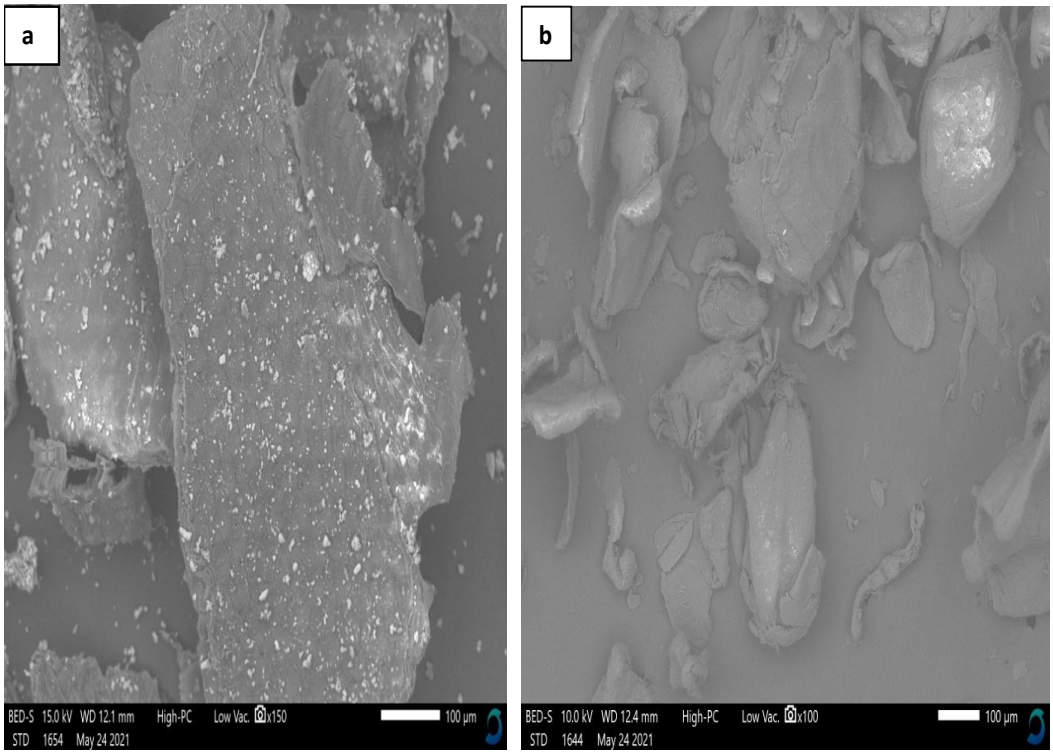


**Plate 4.2*:* Scanning electron micrographs (SEM) of a) Chitosan from BSF pupae shell-chitin obtained through use of chemicals, b) Commercial chitosan**

### 4.5 Antimicrobial properties of chitosan against pathogenic microbes

Chitosan from the black soldier fly exoskeleton exhibited varying degrees of antibacterial activity against *E. coli*, *S. aureus*, *P. aeruginosa*, *B. subtilis*, and *C. albicans,* respectively. The positive control (1% acetic acid) demonstrated antimicrobial activity against all of the tested microbes, whereas the negative control (distilled water) had no inhibitory effects (Plate. 4.3, 4.4 and 4.5). The concentration of BSF chitosan (5, 2.5, 1, and 0.5g/ml) exhibited statistically significant differences ($p \leq 0.005$) in their activities against all the test organisms (Table 4.2).

**Table 4.2: The mean inhibition zones (mm) of black soldier fly chitosan against selected pathogenic microbes**

| **Concentration (g/ml)** | ***C.albicans*** | ***E. coli*** | ***S. aureus*** | ***B. subtilis*** | ***P. aeruginosa*** |
|---|---|---|---|---|---|
| 5 | 26.00±3.61$^{a}$ | 26.33±2.89$^{a}$ | 23.33±3.79$^{a}$ | 24.67±1.53$^{a}$ | 20.33±1.53$^{a}$ |
| 2.5 | 22.00±2.65$^{b}$ | 25.33±2.89$^{a}$ | 21.67±4.93$^{a}$ | 22.67±2.52$^{a}$ | 18.33±1.53$^{a}$ |
| 1 | 16.67±3.21$^{c}$ | 20.00±4.36$^{b}$ | 16.67±2.31$^{b}$ | 16.67±1.53$^{b}$ | 16.67±1.15$^{a}$ |
| 0.5 | 14.33±3.21$^{c}$ | 14.33±3.21$^{c}$ | 13.33±1.53$^{c}$ | 13.67±2.52$^{b}$ | 14.33±0.58$^{b}$ |
| 1% A.A (+Control) | 13.33±1.46$^{c}$ | 11.67±1.46$^{c}$ | 11.67±1.46$^{c}$ | 10.00±1.46$^{c}$ | 12.33±1.46$^{b}$ |
| SDW (-Control) | 0.00±1.46$^{d}$ | 0.00±1.46$^{d}$ | 0.00±1.46$^{d}$ | 0.00±1.46$^{d}$ | 0.00±1.46$^{d}$ |
| P. values | 0.0081 | 0.0087 | 0.0245 | 0.0006 | 0.0023 |

Different letters in the same column indicate significant differences ($p < 0.05$). Values were given as mean ± standard deviation.

There were larger inhibition zones observed at 2.5 and 5 g/ml as compared to 0.5 and 1 g/ml concentrations of chitosan against *E.coli* and *P.aeruginosa*, respectively, while no inhibitions were observed for the negative controls shown (plates 4.3 and 4.4).

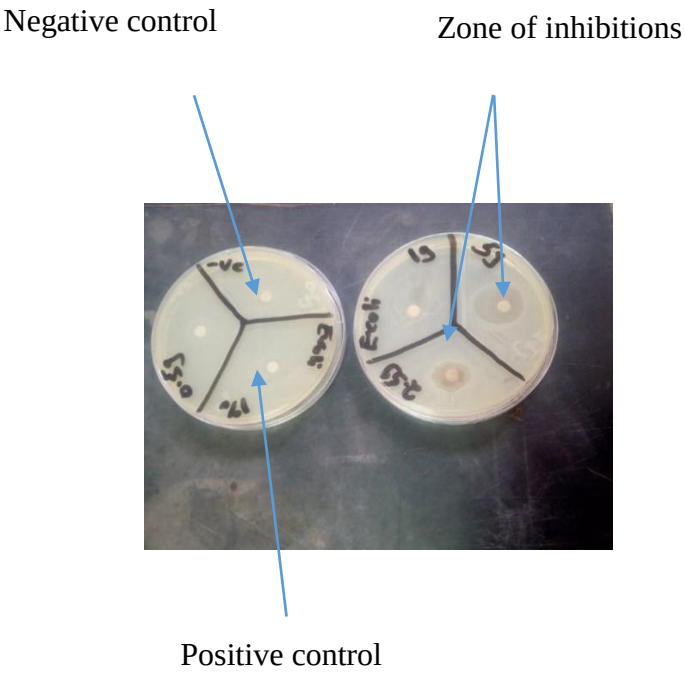


**Plate 4.3: Different concentrations of chitosan (w/v) inhibitions against *E. coli***

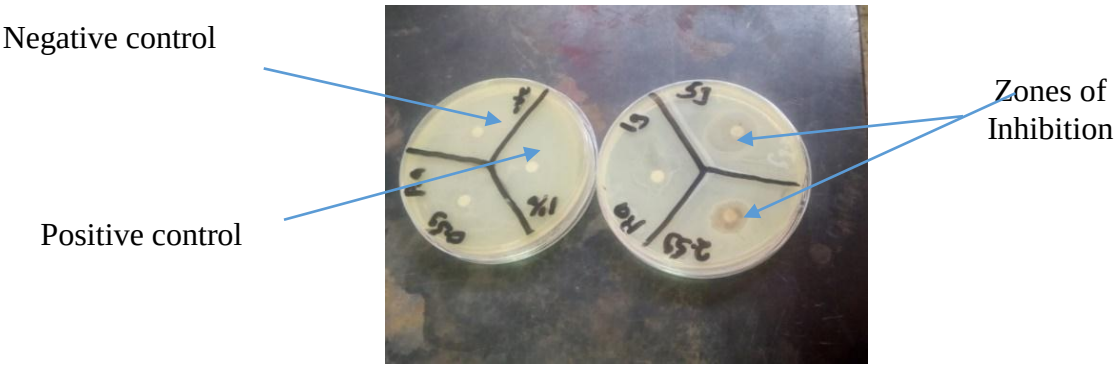


**Plate 4.4: Different concentrations of chitosan (w/v) inhibitions against *P. aeruginosa***

### 4.6 The comparative effects of antimicrobial activities of BSF chitosan, shrimp chitosan and convectional antibiotics against selected pathogenic microbes

There were no significant differences between the activities of chitosan from BSF and commercial shrimp chitosan on *E. coli* (Table 4.3). There was a significant difference ($p \leq 0.005$) on the activities of BSF and commercial shrimp chitosan on *S. aureus, C. albicans, B. subtilis*, and *P. aeruginosa*. On the other hand, the activities of BSF chitosan and nitrofurantoin, streptomycin, cotrimoxazole, and gentamycin on *C. albicans* had no significant difference. Similarly, there were no significant differences between the activities of BSF chitosan and nalidixic acid, tetracycline, and gentamycin on *E. coli*. Other test organisms (*S. aureus, B. subtilis,* and *P. aeruginosa)* demonstrated significant differences between BSF chitosan and all other conventional antibiotics, whereas there were no significant differences between the activities of commercial shrimp chitosan and nitrofurantoin, tetracycline, streptomycin, and nalidixic acid on *S. aureus*. The activities of commercial shrimp chitosan showed no significant differences as compared with nitrofurantoin, tetracycline, and nalidixic acid on *Bacillus subtilis*. Amongst conventional antibiotics, gentamycin was sensitive to all of the tested micro-organisms and demonstrated the highest zones of inhibition in each of the test organisms, with *Bacillus subtilis* exhibiting the highest diameter of inhibition. Ampicillin showed no inhibitory effect on all of the tested microbes. *Bacillus subtilis* and *S. aureus* were resistant to sulphathiazole, streptomycin, and cotrimoxazole, respectively. Sulphathiazole and cotrimoxazole had no inhibitory effects on *P. aeruginosa*. Contrarily, *E. coli* was resistant to ampicillin only while *C. albicans* was not susceptible to ampicillin and nalidixic acid.

Table 4.3: The mean inhibition zones (mm) of conventional antibiotics against selected pathogenic microbes

| **Antibiotics** | ***C.albicans*** | ***E.coli*** | ***S.aureus*** | ***B.subtilis*** | ***P.aeruginosa*** |
|---|---|---|---|---|---|
| BSF chitosan (5 g/ml) | 26.00±3.61$^{a}$ | 26.33±2.89$^{a}$ | 23.33±3.79$^{b}$ | 24.67±1.53$^{b}$ | 20.33±1.53$^{cd}$ |
| Shrimp chitosan (5g/ml) | 23.67±1.53$^{a}$ | 23.67±3.21$^{a}$ | 23.33±4.93$^{b}$ | 24.67±2.52$^{b}$ | 18.67±1.15$^{d}$ |
| Gentamycin (10 mcg) | 26.33±1.53$^{a}$ | 26.33±1.53$^{a}$ | 28.00±1.00$^{a}$ | 30.00±1.00$^{a}$ | 30.00±1.00$^{a}$ |
| Nalidixic acid (30 mcg) | 25.33±1.53$^{a}$ | 25.33±1.53$^{a}$ | 21.33±0.58$^{bc}$ | 21.00±1.00$^{c}$ | 22.33±2.08$^{b}$ |
| Tetracycline (100 mcg) | 19.00±1.00$^{b}$ | 19.00±1.00$^{b}$ | 18.00±1.00$^{c}$ | 24.33±0.58$^{b}$ | 20.00±1.00$^{d}$ |
| Nitrofurantoin (200 mcg) | 15.33±1.53$^{c}$ | 15.33±1.53$^{c}$ | 19.00±1.00$^{c}$ | 18.33±0.58$^{d}$ | 24.00±2.00$^{b}$ |
| Streptomycin (25 mcg) | 15.00±1.00$^{c}$ | 15.00±1.00$^{c}$ | 18.00±1.00$^{c}$ | 0.00±0.00$^{e}$ | 14.67±0.58$^{e}$ |
| Sulphathiazole (200 mcg) | 11.00±1.00$^{d}$ | 11.00±1.00$^{d}$ | 0.00±0.00$^{d}$ | 0.00±0.00$^{e}$ | 0.00±0.00$^{f}$ |
| Cotrimoxazole (25 mcg) | 11.00±1.00$^{d}$ | 11.00±1.00$^{d}$ | 0.00±0.00$^{d}$ | 0.00±0.00$^{e}$ | 0.00±0.00$^{f}$ |
| Ampicillin (25 mcg) | 0.00±0.00$^{e}$ | 0.00±0.00$^{e}$ | 0.00±0.00$^{d}$ | 0.00±0.00$^{e}$ | 0.00±0.00$^{f}$ |
| **P-Values** | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| **L.S.D values** | 2.76 | 2.93 | 3.53 | 1.81 | 2.04 |

Treatments with the same letter are not significantly different. Values were given as mean ± standard deviation.

There were larger inhibition zones observed at 2.5 and 5 g/ml concentrations of chitosan against *S.aureus* and *E.coli*, while amongst antibiotics, larger inhibitions were observed for gentamycin, tetracycline, and nalidixic acid against *S.aureus* and *E.coli*, as shown on plate 4.5.

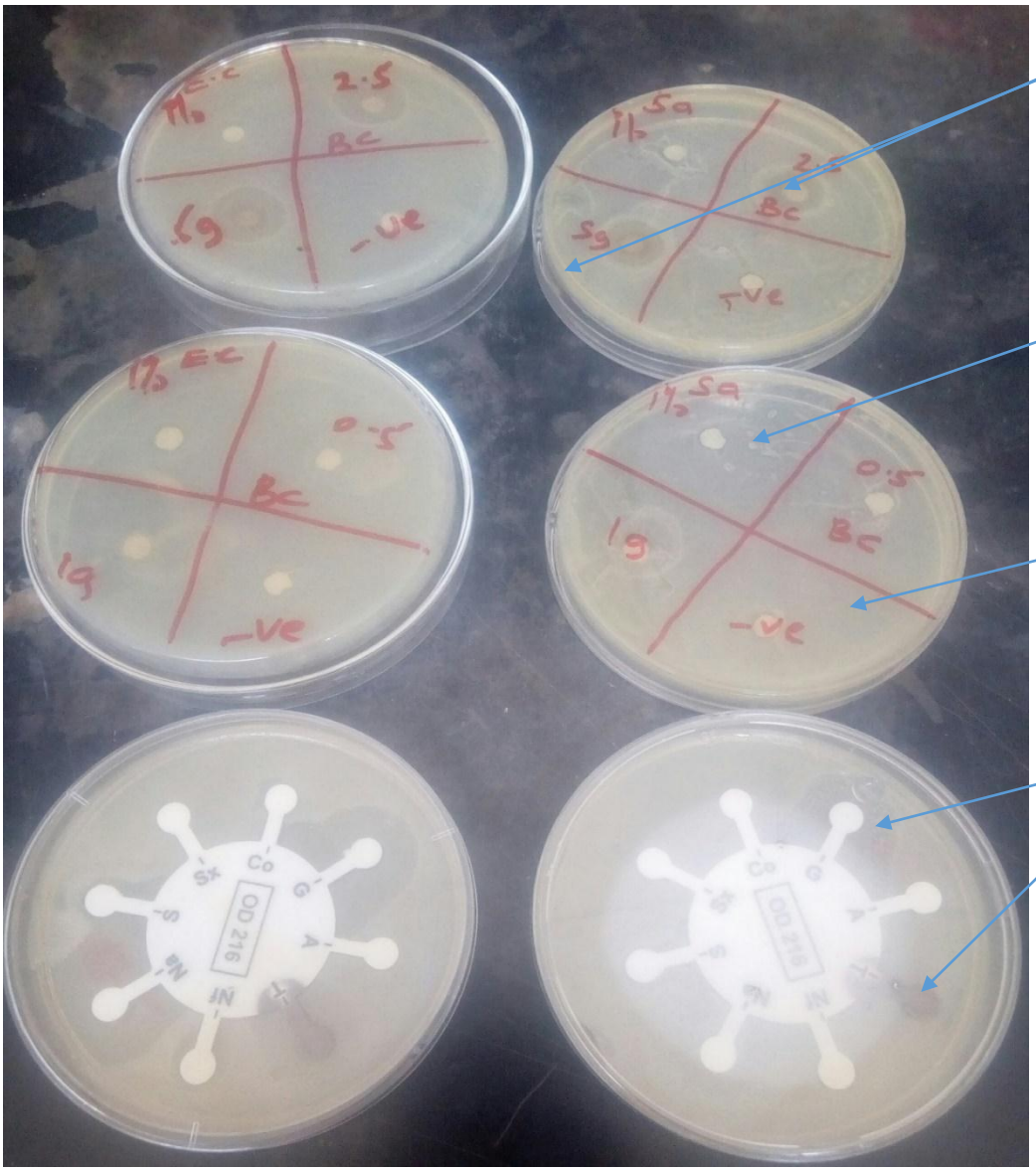


**Plate 4.5: Commercial antibiotics and different concentrations of chitosan (w/v) inhibitions against *S. aureus* and *E. coli***

# CHAPTER FIVE

# DISCUSSIONS

## 5.1 Chitin yield from the exoskeleton of the black soldier fly

The chitin yield obtained from Black soldier fly exoskeleton through both biological and chemical means was higher than the yield obtained from the findings reported by (Caligiani *et al*., 2018; Spranghers *et al*., 2017; Wong *et al*., 2019). The difference could have been due to the use of clean exoskeletons in this study, while previous studies used dead flies (Złotko *et al*., 2021). The sequence of extraction, deproteinization (DP) then demineralization (DM) led to a decrease in chitin yield, this was in line with the findings reported (Zimri, 2018). This was because DP, before DM, eroded the protein layer that covered the chitin matrix, exposing it to acidic treatment and causing significant inorganic material removal. Significant hydrolysis and loss of chitin fraction resulted in a low yield of chitin (Zimri, 2018). The finding on chitin yield obtained in this study through biological means differed from the results obtained from crabs where they found a higher chitin yield (Hajji *et al*., 2014). This variation was due to different sources of chitin, such as marine, fungal, and insects (Hahn *et al*., 2020). The results obtained from this study can be compared with the findings of yields from other insects, for example, grasshoppers, beetles, and daphnia (Erdogan & Kaya *et al.*, 2016; Marei *et al.*, 2016; Sargin *et al*., 2019). In this study, the chitosan yield obtained from the BSF exoskeleton (6.58%) through chemical means differed with the findings by (Zimri, 2018). The variation in chitosan yield may have been due to excessive depolymerisation of the chitosan polymer and loss of the sample due to excessive removal of acetyl groups during deacetylation (Zimri, 2018). The chitosan yield obtained was also comparable to other sources like the cockroach, shrimp, *Musca domestica* and krill (Wanule, 2014; Hossain & Iqbal, 2014; Kim *et al*., 2016; Yuan *et al*., 2020).

## 5.2 Functional groups of chitin extracted from the Black Soldier fly exoskeleton

The band at 3440 $cm^{-1}$ was broad and was due to the presence of hydroxyl groups in chitin. The band at 2355 $cm^{-1}$ was attributed to the presence of CH stretching vibrations, which were indicative of the presence of methyl groups. Additionally, the band at 1650 $cm^{-1}$ was attributed to C=O secondary amide I stretching vibrations and the band at 1550 $cm^{-1}$ was attributed to amide II, which was indicative of an N-H bend.

These characteristic peaks were similar to the findings made on chitin from BSF larvae and adult flies (Zimri, 2018). These results were also in line with the studies done on chitin from grasshoppers that got the same characteristic bands (Erdogan & Kaya, 2016). This study revealed that the chitin extracted from the BSF pupae exoskeleton through biological and chemical means was of α-form and was very similar to that of commercial shrimp chitin. Alpha (α-) chitin had characteristic chitin peaks recorded at 1650, 1620, and 1550 $cm^{-1}$ (Erdogan & Kaya, 2016).

In addition, the presence of the glycosidic bond at wavenumber 896 $cm^{-1}$ was a characteristic band for an alpha, which was detected in all chitin samples (Tsurkan *et al*., 2020). It was found that there was no difference between the structures of biological, chemical, and shrimp chitin. In all the chitin samples, there were no peaks at 1540 $cm^{-1}$. The absence of bands at 1540 $cm^{-1}$ could be attributed to the absence of protein contaminants showing sufficient deproteination (Hassainia *et al*., 2018).

Other major bands detected were 1413 $cm^{-1}$ ($CH_2$ ending and $CH_3$ deformation), 1256 $cm^{-1}$ (CH bends and $CH_3$ symmetrical deformation), 1069 $cm^{-1}$ (Asymmetric in the phase ring stretching mode), 1023 $cm^{-1}$ (C-O-C asymmetric stretch in phase ring), 1159 $cm^{-1}$ ($CH_2$ wagging), 1114 $cm^{-1}$ (Asymmetric bridge oxygen stretching), and 896 $cm^{-1}$ (glyosidic bond). The results obtained blended well with the previous studies (Kaya-Erdogan Mol *et al*., 2015; Zimri, 2018).

### 5.3 Functional groups of chitosan extracted from the Black Soldier fly exoskeleton

These characteristic chitosan findings obtained in this study were in line with those reported on chitosan from BSF larvae and adult flies, which reported similar characteristic peaks by (Zimri, 2018). The peaks at around 1650-1655 $cm^{-1}$ and 1583-1590 $cm^{-1}$, corresponded to (C=O) in the $NHCOCH_3$ group (amide I band) and ($NH_2$) in the $NHCOCH_3$ group (amide II band), were characteristic of chitosan (Zimri, 2018).

Additional broad absorption bands observed at 3250-3750 $cm^{-1}$ were attributed to symmetric stretching vibrations of the O-H and NH groups caused by the strong intermolecular hydrogen bonding of chitosan polysaccharides. The peaks at approximately 2350 $cm^{-1}$ were attributed to symmetric and asymmetric vibrations of C-H groups in the chitosan samples. The absorption peaks at 1374 and 1255 $cm^{-1}$ were ascribed to N-H bending vibrations of primary amides and C-O-C stretching vibrations in both chitosan samples. These findings were consistent with those obtained using chitosan derived from grasshopper species (Erdogan & Kaya, 2016). The peaks displayed at around 1153 $cm^{-1}$ and 1082 $cm^{-1}$ were attributed to the β (1-4) glyosidic bond in the polysaccharide unit and stretching vibrations of C-O-C in the glucose ring. These findings were similar to those obtained using chitosan derived from shrimp shells (Eddya *et al*., 2020). The absorption band of amide II had a lower intensity than that of amide I, which suggested effective deacetylation. When chitin was converted to chitosan, the intensity of the amide II absorption band decreased while the intensity of amide I increased, which showed the formation of amide ($NH_2$) groups. The FTIR results suggested that there was a similarity between the chemical composition and bonding types of chitosan in the pupae shells and commercial shrimp chitosan (Figure 4.2). These findings were similar to those obtained using chitosan from nymphs and adult grasshoppers (Erdogan & Kaya, 2016).

### 5.4 The surface morphology of chitin and chitosan from Black soldier fly exoskeleton

The findings in this study on chitosan from BSF pupae exoskeleton morphology were in agreement with previous studies done on BSF chitosan from pupae shells (Kaya *et al*., 2016; Zimri, 2018). Commercial shrimp chitosan had a dense, smooth surface without fibers and/or pores. This finding differed from the observations of previous studies (Ibitoye *et al*., 2018; Marei *et al*., 2016; Zimri, 2018). An increase in magnification X1000 revealed that all the biologically extracted chitins had fibers and pores, but low magnification gave clearer micrographs. Chitin obtained by chemical means had a smooth surface without fibers and pores, and the surface morphologies were tightly packed structures with repeating circular and hexagonal units in a honeycomb-like arrangement (Plate. 4.2 d). These observations were similar to studies done on BSF pupae chitin (Kaya-Asan-Ozusaglam *et al*., 2017; Zimri, 2018) who also found the chitins to have a surface morphologies were tightly packed structures with repeating circular and hexagonal units in a honeycomb-like arrangement .

Commercial shrimp chitin had a rocky smooth surface, without fibers, and numerous pores of 100 µm in diameter at X500 magnification (Plate 4.2 e), which was similar to studies done earlier by (Ibitoye *et al*., 2018; Marei *et al*., 2016; Zimri, 2018). They found shrimp chitin to have a rocky smooth surface, without fibers, and numerous pores of 100 µm in diameter at X500 magnification. BSF pupae exoskeleton chitosan obtained through chemical means had a rough surface, without any fibers and/or pores on both surfaces (Plate.4.2 a), while commercial shrimp chitosan had a rocky smooth surface without fibers and pores (Plate.4.2 b). According to the literature, chitin obtained from various sources has different morphologies (Al Sagheer *et al*., 2009). The surface morphology of biologically extracted chitins from the BSF pupae exoskeleton in this study was similar to that of other organisms such as crabs, grasshoppers, potato beetles, locusts, and house crickets (Erdogan & Kaya, 2016; Ibitoye *et al*., 2018; Kaya-Erdogan Mol *et al*., 2015; Marei *et al*., 2016; Al Sagheer *et al*., 2009).

The surface morphology of the chemically extracted chitin from the BSF pupae exoskeleton obtained was similar to other sources, such as fungal sources like *L. vellereus* and *P. ribis* (Erdogan *et al*., 2017) and lichen species such as *X. parietina* (Kaya-Halici *et al*., 2015). Surface morphology plays an effective role in determining the use of chitin in different fields, which has been emphasized by many researchers (Hajji *et al*., 2014; Al Sagheer *et al*., 2009; Zimri, 2018). For example, chitin with a porous structure has been employed in the absorption of toxic metal ions and has been used in controlled drug delivery and tissue engineering, while chitin with a fibrous structure is used in textiles (Wang *et al*., 2020).

### 5.5 Antimicrobial properties of chitosan against pathogenic microbes

The inhibitory activity increased with the increase in the concentration of chitosan in each of the tested microbes. Black soldier fly chitosan showed the highest inhibition zone against *Candida albicans*. These findings contrasted with the findings on chitosan from grasshopper species and BSF, respectively (Kaya-Asan-Ozusaglam *et al*., 2017; Leke-Aladekoba, 2018).

In this study, chitosan inhibited the growth of *E. coli*, and these findings were similar to the previous studies on the activity of chitosan on *E. coli* (Al-Nabulsi *et al*., 2020; Elshaarawy *et al*., 2020). Chitosan from *Hermetia illucens* showed stronger antibacterial activity against *S. aureus* as compared to the observations from previous studies (Divya *et al*., 2017; Elshaarawy *et al*., 2020; Kaya-Asan-Ozusaglam *et al*., 2017). Black soldier fly chitosan demonstrated stronger activity against *P. aeruginosa* compared to the findings by (Khalil *et al*., 2017; Umoren *et al*., 2020). Chitosan from BSF demonstrated notable antibacterial activity against *B. subtilis* bacteria, which was in line with previous studies (Alves *et al*., 2020). Black soldier fly chitosan also showed a stronger antifungal property as demonstrated by great inhibition of *C.albicans*. This was similar to the studies done by (Kaya, Baran *et al*., 2015; Khan *et al*, 2020; Namangkalakul *et al*., 2020). The chitosan activity on *Candida albicans* was higher than in previous studies (Khalil *et al*., 2017).

### 5.6 The comparative effects of antimicrobial activities of BSF chitosan, shrimp chitosan and convectional antibiotics against selected pathogenic microbes

There was no significant difference between the activities of BSF chitosan and commercial shrimp chitosan on all the tested organisms (*E. coli, B. subtilis, P. aeruginosa, C. albicans,* and *S. aureus*). These results obtained were consistent with previous studies which reported activities of commercial and BSF chitosan to have antimicrobial activities against, *E. coli*, *B. subtilis*, *P. aeruginosa*, *C. albicans*, and *S. aureus* respectively (de Souza Vilela *et al*., 2021; Vandeweyer *et al*., 2020; Jamróz & Kopel, 2020; Lagat *et al*., 2021; Nair *et al*., 2022). *C. albicans* was highly susceptible to the effects of BSF chitosan, shrimp chitosan, nalidixic acid, and gentamycin as it inhibited the growth of *C. albicans*. Conversely, *Candida albicans* was resistant to the effects of BSF chitosan, commercial shrimp chitosan, ampicillin, tetracycline, nitrofurantoin, streptomycin, cotrimoxazole, and sulphathiazole. This observation was in agreement with those who reported the effects of ampicillin on *Candida albicans* (Kaya-Asan-Ozusaglam *et al*., 2017). *E. coli* was susceptible to the activities of BSF chitosan, commercial shrimp chitosan, nalidixic acid, and gentamycin. The results obtained were in agreement with the previous studies reported by (Kemboi *et al*., 2022) who also found *E.coli* susceptible to nalidixic acid and gentamycin. *P.aeruginosa* was resistant to the activities of BSF chitosan, commercial shrimp chitosan, ampicillin, tetracycline, nitrofurantoin, streptomycin, sulphathiazole, and clotrimazole. This present study, conquered with previous studies reported by (Lagat *et al*., 2021) who also found *P. aeruginosa* resistant to ampicillin, tetracycline, nitrofurantoin, streptomycin, sulphathiazole, and clotrimazole. On the other hand, *S.aureus* was susceptible to the activities of BSF chitosan, commercial shrimp chitosan, and nalidixic acid. However, *S.aureus* was resistant to the activities of BSF chitosan, commercial shrimp chitosan, ampicillin, tetracycline, nitrofurantoin, streptomycin, sulphathiazole, cotrimoxazole, and gentamycin.

These results were consistent with previous studies reported (Nair *et al*., 2022; Zare *et al*., 2019; Shariatinia, 2020). *Bacillus subtilis* was susceptible to the activities of BSF chitosan, shrimp chitosan, and tetracycline.

However, *B.subtilis* was resistant to the activities of BSF chitosan, shrimp chitosan, nitrofurantoin, streptomycin, cotrimoxazole, sulphathiazole, gentamycin, and nalidixic acid and these results were in line with the previous studies reported by (Lagat *et al*., 2021) who also found out *B.subtilis* resistant to nitrofurantoin, streptomycin, cotrimoxazole, sulphathiazole, gentamycin, and nalidixic acid. *P.aeruginosa* was found to be susceptible to the activities of BSF chitosan, commercial chitosan, and nitrofurantoin on *P.aeruginosa*. However, *P. aeruginosa* was resistant to the activities of BSF chitosan, commercial shrimp chitosan, ampicillin, nalidixic acid, streptomycin, tetracycline, sulphathiazole, cotrimoxazole, and gentamycin. All of test organisms were resistant to ampicillin also *S. aureus*, *B.subtilis*, and *P.aeruginosa* were resistant to sulphathiazole, and cotrimoxazole all this were attributed to the resistance of the test organisms. Both BSF and shrimp-derived chitosan were found to be more effective against gram-negative bacteria than gram-positive bacteria. This could have been ascribed to the fact that chitosan alters cell permeability by deposition of chitosan on the pathogen cell surface, resulting in the formation of an impermeable polymeric layer that inhibited nutrient uptake in the cell and changed the metabolite secretion in the extracellular matrix (Chandrasekaran *et al*., 2020; Youssef & Hashim, 2020).The results obtained were similar to previous studies (Chandrasekaran *et al*., 2020; Choi *et al*., 2012; Hipalaswins *et al*., 2016; Kaya-Asan-Ozusaglam *et al*., 2017) where they demonstrated the high activity of chitosan against gram-negative as opposed to gram-positive bacteria these may be attributed to high concentration of positive charges in the chitosan structure may have created significant electrostatic interactions with negatively charged residues of carbohydrates, proteins, and lipids present in microbial cells, impeding bacterial proliferation (Homaeigohar & Boccaccini, 2020).

This study, however, differed from previous studies, which demonstrated stronger activity against gram-positive bacteria as opposed to gram-negative bacteria (Abdallah *et al*., 2020; Shapi'i *et al*., 2020; Silva *et al*., 2020).

# CHAPTER SIX

# CONCLUSIONS AND RECOMMENTATIONS

## 6.1 Conclusions

1. This research demonstrated that chitin could effectively be extracted from the BSF exoskeleton by co-cultivating *P. aeruginosa* and *B. subtilis* bacteria. Biological extraction gave higher yields as compared to chemical extraction and can be used as an alternative method since it is inexpensive and eco-friendly.
2. The FTIR results showed that there was a close similarity in bonding and the chemical structure of all the chitin samples and that they were of the alpha form (α). Scanning electron microscopy revealed that the surface morphologies of biologically extracted chitins consisted of fibers and pores together, while chemically extracted chitins had neither fibers nor pores. This showed that biologically derived BSF chitin could therefore be used in place of a chemically derived BSF chitin.
3. Chitosan from BSF exhibited activity against all of the tested pathogenic microbes and could be used as a novel antibiotic compound for combating antibiotic-resistant strains.

## 6.2 Recommendations

1) There is a need to demonstrate the mode of action of chitosan on various microbes since the killing mechanism of chitosan has not been thoroughly researched.
2) An electrospinning approach to generating nanofibers made from chitosan with a substantially high yield suitable for scale-up production should be the ultimate goal to push electrospun nanofibers from the laboratory to the industry.
3) There is need for establishing the effect of chitosan on non-target organisms as a requirement in the product development process.
4) There is need for future studies to determine the use of chitosan in fields like agriculture, waste water treatment and wound healing among other sectors.

## REFERENCES


Abd El-Hack, M. E., Shafi, M. E., Alghamdi, W. Y., Abdelnour, S. A., Shehata, A. M., Noreldin, A. E., ... & Ragni, M. (2020). Black soldier fly (*Hermetia illucens*) meal as a promising feed ingredient for poultry: A comprehensive review. *Agriculture*, *10*(8), 339.

Abdallah, O. M., EL-Baghdady, K. Z., Khalil, M. M., El Borhamy, M. I., & Meligi, G. A. (2020). Antibacterial, antibiofilm and cytotoxic activities of biogenic polyvinyl alcohol-silver and chitosan-silver nanocomposites. *Journal of Polymer Research*, *27* (3), 1-9.'

Ahing, F. A., & Wid, N. (2016). Extraction and characterization of chitosan from shrimp shell waste in sabah. *Transactions on Science and Technology*, 3(12), 227–237.

Ahmed, A. A., Sofy, A. R., & Sharaf, A. M. A. (2017). Effectiveness of chitosan as naturally-derived antimicrobial to fulfil the needs of today's consumers looking for food without hazards of chemical preservatives. *Microbiol RES J*, *7*, 55-67.

Akhtar, K., Khan, S. A., Khan, S. B., & Asiri, A. M. (2018). Scanning electron microscopy: principle and applications in nanomaterials characterization. In *Handbook of materials characterization* (pp. 113-145). Springer, Cham.

Akram, K., Ahn, J. J., & Kwon, J. H. (2012). Identification and Characterization of Gamma- Irradiated Dried *Lentinus edodes* Using ESR, SEM, and FTIR Analyses. *Journal of Food Science*, *77*(6), 690-C696.

Al Sagheer, F. A., Al-Sughayer, M. A., Muslim, S., & Elsabee, M. Z. (2009). Extraction and characterization of chitin and chitosan from marine sources in Arabian Gulf. *Carbohydrate polymers*, *77*(2), 410-419.

Al-Nabulsi, A., Osaili, T., Sawalha, A., Olaimat, A. N., Albiss, B. A., Mehyar, G., ... & Holley, R. (2020). Antimicrobial activity of chitosan coating containing ZnO nanoparticles against *E. coli* O157: H7 on the surface of white brined cheese. *International Journal of Food Microbiology*, *334*, 108838.

Alves, T. F., Morsink, M., Batain, F., Chaud, M. V., Almeida, T., Fernandes, D. A., ... & Severino, P. (2020). Applications of natural, semi-synthetic, and synthetic polymers in cosmetic formulations. *Cosmetics*, *7*(4), 75.

Aminzadeh, M., Aminossadati, S. M., Kizil, M. S., & Rakić, A. D. (2018). Recent developments in fibre optic shape sensing. *Measurement*, *128*, 119-137.

Aytekin, O., & Elibol, M. (2010). Cocultivation of *Lactococcus lactis* and *Teredinobacter turnirae* for biological chitin extraction from prawn waste. *Bioprocess and Biosystems Engineering*, *33*(3), 393-399.

Barnes, K. M., Gennard, D. E., & Dixon, R. A. (2010). An assessment of the antibacterial activity in larval excretion/secretion of four species of insects recorded in association with corpses, using *Lucilia sericata* Meigen as the marker species. *Bulletin of entomological research*, *100*(6), 635-640.

Barragan-Fonseca, K. B., Dicke, M., & van Loon, J. J. (2017). Nutritional value of the black soldier fly (*Hermetia illucens* L.) and its suitability as animal feed–a review. *Journal of Insects as Food and Feed*, *3*(2), 105-120.

Benelli, G., Canale, A., Raspi, A., & Fornaciari, G. (2014). The death scenario of an Italian Renaissance princess can shed light on a zoological dilemma: did the black soldier fly reach Europe with Columbus? *Journal of Archaeological Science*, 49, 203–205.

Blair, J. M., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. (2015). Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology*, *13*(1), 42-51.

Boarin Alcalde, L., & Graciano Fonseca, G. (2016). Alkali process for chitin extraction and chitosan production from Nile tilapia (*Oreochromis niloticus*) scales. *Latin American Journal of Aquatic Research*, 44(4), 683–688.

Caligiani, A., Marseglia, A., Leni, G., Baldassarre, S., Maistrello, L., Dossena, A., & Sforza, S. (2018). Composition of black soldier fly prepupae and systematic approaches for extraction and fractionation of proteins, lipids and chitin. *Food research international*, *105*, 812-820.

Casadidio, C., Peregrina, D. V., Gigliobianco, M. R., Deng, S., Censi, R., & Di Martino, P.

(2019). Chitin and Chitosan: Characteristics, Eco-Friendly Processes, and Applications in Cosmetic Science. *Marine drugs*, *17*(6), 369.

Chandrasekaran, M., Kim, K. D., & Chun, S. C. (2020). Antibacterial activity of chitosan nanoparticles: A review. *Processes*, *8*(9), 1173.

Chen, W., Wang, C., Gao, Y., Wu, Y., Wu, G., Shi, X., ... & Deng, H. (2020). Incorporating chitin derived glucosamine sulfate into nanofibers via coaxial electrospinning for cartilage regeneration. *Carbohydrate polymers*, *229*, 115544.

Choi, W. H., Yun, J. H., Chu, J. P., & Chu, K. B. (2012). Antibacterial effect of extracts of *Hermetia illucens* (Diptera: *Stratiomyidae*) larvae against gram- negative bacteria. *Entomological research*, *42*(5), 219-226.

Confederat, L. G., Tuchilus, C. G., Dragan, M., Sha'at, M., & Dragostin, O. M. (2021). Preparation and antimicrobial activity of chitosan and its derivatives: A concise review. *Molecules*, *26*(12), 3694.

Cui, H., Surendhiran, D., Li, C., & Lin, L. (2020). Biodegradable zein active film containing chitosan nanoparticle encapsulated with pomegranate peel extract for food packaging. *Food Packaging and Shelf Life*, *24*, 100511.

Daraghmeh, N. H., Chowdhry, B. Z., Leharne, S. A., Al Omari, M. M., & Badwan, A. A. (2011). Chitin. In profiles of drug substances, excipients, and related methodology, 36, 35–10.

de Souza Vilela, J., Andronicos, N. M., Kolakshyapati, M., Hilliar, M., Sibanda, T. Z., Andrew, N. R., ... & Ruhnke, I. (2021). Black Soldier Fly larvae in broiler diets improve broiler performance and modulate the immune system. *Animal Nutrition*, *7*(3), 695-706.

Divya, K., Vijayan, S., George, T. K., & Jisha, M. S. (2017). Antimicrobial properties of chitosan nanoparticles: Mode of action and factors affecting activity. *Fibers and polymers*, *18*(2), 221-230.

Eddya, M., Tbib, B., & Khalil, E. H. (2020). A comparison of chitosan properties after extraction from shrimp shells by diluted and concentrated acids. *Heliyon*, *6*(2), e03486.

Elshaarawy, R. F., Ismail, L. A., Alfaifi, M. Y., Rizk, M. A., Eltamany, E. E., & Janiak, C. (2020). Inhibitory activity of biofunctionalized silver-capped N-methylated water-soluble chitosan thiomer for microbial and biofilm infections. *International journal of biological macromolecules*, *152*, 709-717.

Erdogan, S., & Kaya, M. (2016). High similarity in physicochemical properties of chitin and chitosan from nymphs and adults of a grasshopper. *International journal of biological macromolecules*, *89*, 118-126.

Erdogan, S., Kaya, M., & Akata, I. (2017). Chitin extraction and chitosan production from cell wall of two mushroom species (*Lactarius vellereus* and *Phyllophora ribis*). In *AIP Conference Proceedings 1809*(1), 020012.

Fuster, M. G., Montalbán, M. G., Carissimi, G., Lima, B., Feresin, G. E., Cano, M., ... & Víllora, G. (2020). Antibacterial Effect of Chitosan–Gold Nanoparticles and Computational Modeling of the Interaction between Chitosan and a Lipid Bilayer Model. *Nanomaterials*, *10*(12), 2340.

Gabler, F., Lalander, C., & Vinnerås, B. (2014). Using black soldier fly for waste recycling and effective Salmonella spp. reduction. Agronomy for sustainable development, 35, 261-271.

Gao, Y., Xu, Z., Chen, S., Gu, W., Chen, L., & Li, Y. (2008). Arginine-chitosan/DNA self-assemble nanoparticles for gene delivery: In vitro characteristics and transfection efficiency. *International journal of pharmaceutics*, *359*(1-2), 241-246.

Gholinia, A., Curd, M. E., Bousser, E., Taylor, K., Hosman, T., Coyle, S., ... & Withers, P. J. (2020). Coupled Broad Ion Beam–Scanning Electron Microscopy (BIB–SEM) for polishing and three-dimensional (3D) serial section tomography (SST). *Ultramicroscopy*, *214*, 112989.

Hahn, T., Tafi, E., Paul, A., Salvia, R., Falabella, P., & Zibek, S. (2020). Current state of chitin purification and chitosan production from insects. *Journal of Chemical Technology & Biotechnology*, *95*(11), 2775-2795.

Hajji, S., Younes, I., Ghorbel-Bellaaj, O., Hajji, R., Rinaudo, M., Nasri, M., & Jellouli, K. (2014). Structural differences between chitin and chitosan extracted from three different marine sources. *International journal of biological macromolecules*, *65*, 298-306.

Hassainia, A., Satha, H., & Boufi, S. (2018). Chitin from *Agaricus bisporus*: Extraction and characterization. *International journal of biological macromolecules*, *117*, 1334-1342.

Hipalaswins, W. M., Balakumaran, M., & Jagadeeswari, S. (2016). Synthesis, characterization and antibacterial activity of chitosan nanoparticles and its impact on seed germination. *J. Acad. Ind. Res*, *5*, 65.

Homaeigohar, S., & Boccaccini, A. R. (2020). Antibacterial biohybrid nanofibers for wound dressings. *Acta biomaterialia*, *107*, 25-49.

Hossain, M. S., & Iqbal, A. (2014). Production and characterization of chitosan from shrimp waste. *Journal of the Bangladesh Agricultural University*, *12*(1), 153-160.

Ibitoye, E. B., Lokman, I. H., Hezmee, M. N. M., Goh, Y. M., Zuki, A. B. Z., & Jimoh, A. A. (2018). Extraction and physicochemical characterization of chitin and chitosan isolated from house cricket. *Biomedical Materials*, *13*(2), 025009.

Inbaraj, B. S., Chen, B. Y., Liao, C. W., & Chen, B. H. (2020). Green synthesis, characterization and evaluation of catalytic and antibacterial activities of chitosan, glycol chitosan and poly (γ-glutamic acid) capped gold nanoparticles. *International Journal of Biological Macromolecules*, *161*, 1484-1495.

Islam, S., Bhuiyan, M. A. R., & Islam, M. N. (2017). Chitin and Chitosan: Structure, Properties and Applications in Biomedical Engineering. *Journal of Polymers and the Environment*, *25* (3), 854–866.

Jamróz, E., & Kopel, P. (2020). Polysaccharide and protein films with antimicrobial/antioxidant activity in the food industry: a review. *Polymers*, *12*(6), 1289.

Johney, J., Eagappan, K., & Ragunathan, R. R. (2017). Microbial Extraction of chitin and chitosan from *Pleurotus* spp, its characterization and antimicrobial activity. *Int J Curr Pharm Res*, *9*(1), 88-93.

Joseph, S. M., Krishnamoorthy, S., Paranthaman, R., Moses, J. A., & Anandharamakrishnan, C. (2021). A review on source-specific chemistry, functionality, and applications of chitin and chitosan. *Carbohydrate Polymer Technologies and Applications*, *2*, 100036.

Kansal, D., Hamdani, S. S., Ping, R., Sirinakbumrung, N., & Rabnawaz, M. (2020). Food-safe chitosan–zein dual-layer coating for water-and oil-repellent paper substrates. *ACS Sustainable Chemistry & Engineering*, *8*(17), 6887-6897.

Kaur, S., & Dhillon, G. S. (2015). Recent trends in biological extraction of chitin from marine shell wastes: a review. *Critical reviews in biotechnology*, *35*(1), 44-61.

Kaya, M., Baran, T., & Karaarslan, M. (2015). A new method for fast chitin extraction from shells of crab, crayfish and shrimp. *Natural Product Research*, *29*(15), 1477-1480.

Kaya, M., Baran, T., Asan-Ozusaglam, M., Cakmak, Y. S., Tozak, K. O., Mol, A., ... & Sezen, G. (2017). Extraction and characterization of chitin and chitosan with antimicrobial and antioxidant activities from cosmopolitan Orthoptera species (*Insecta*). *Biotechnology and Bioprocess Engineering*, *20*(1), 168-179.

Kaya, M., Baran, T., Erdoŧan, S., Menteş, A., Aşan Özüsaŧlam, M., & Çakmak, Y. S. (2014). Physicochemical comparison of chitin and chitosan obtained from larvae and adult Colorado potato beetle (*Leptinotarsa decemlineata*). *Materials Science and Engineering* C, 45, 72–81.

Kaya, M., Erdogan, S., Mol, A., & Baran, T. (2015). Comparison of chitin structures isolated from seven Orthoptera species. *International journal of biological macromolecules*, *72*, 797-805.

Kaya, M., Halıcı, M. G., Duman, F., Erdoğan, S., & Baran, T. (2015). Characterisation of α-chitin extracted from a lichenised fungus species *Xanthoria parietina*. *Natural product research*, *29*(13), 1280-1284.

Kaya, M., Sofi, K., Sargin, I., & Mujtaba, M. (2016). Changes in physicochemical properties of chitin at developmental stages (larvae, pupa and adult) of *Vespa crabro* (wasp). *Carbohydrate polymers*, *145*, 64-70.

Kemboi, V. J., Kipkoech, C., Njire, M., Were, S., Lagat, M. K., Ndwiga, F., ... & Tanga, C. M. (2022). Biocontrol Potential of Chitin and Chitosan Extracted from Black Soldier Fly Pupal Exuviae against Bacterial Wilt of Tomato. *Microorganisms*, *10*(1), 165.

Khalil, A. M., Abdel-Monem, R. A., Darwesh, O. M., Hashim, A. I., Nada, A. A., & Rabie, S. T. (2017). Synthesis, characterization, and evaluation of antimicrobial activities of chitosan and carboxymethyl chitosan schiff-base/silver nanoparticles. *Journal of Chemistry*, *2017*.

Khan, S. H., Younus, H., Allemailem, K. S., Almatroudi, A., Alrumaihi, F., Alruwetei, A. M., ... & Khan, M. A. (2020). Potential of methylglyoxal-conjugated chitosan nanoparticles in treatment of fluconazole-resistant *Candida albicans* infection in a murine model. *International journal of nanomedicine*, *15*, 3681.

Khayrova, A., Lopatin, S., & Varlamov, V. (2020). Obtaining chitin/chitosan-melanin complexes from black soldier fly *Hermetia illucens*. In *IOP Conference Series: Materials Science and Engineering 809*(1), 012020.

Kim, M. W., Han, Y. S., Jo, Y. H., Choi, M. H., Kang, S. H., Kim, S. A., & Jung, W. J. (2016). Extraction of chitin and chitosan from housefly, *Musca domestica*, pupa shells. *Entomological Research*, *46*(5), 324-328.

Kipkoech, C., Kinyuru, J. N., Imathiu, S., & Roos, N. (2017). Use of house cricket to address food security in Kenya: Nutrient and chitin composition of farmed crickets as influenced by age. *African Journal of Agricultural Research*, *12*(44), 3189-3197.

Kourmouli, A., Valenti, M., van Rijn, E., Beaumont, H. J., Kalantzi, O. I., Schmidt-Ott, A., & Biskos, G. (2018). Can disc diffusion susceptibility tests assess the antimicrobial activity of engineered nanoparticles? *Journal of Nanoparticle Research*, *20*(3), 1-6.

Kurita, K. (2020). Chemical modifications of chitin and chitosan. In *Chitin in nature and technology* (pp. 287-293). Springer, Boston, MA.

Lagat, M. K., Were, S., Ndwigah, F., Kemboi, V. J., Kipkoech, C., & Tanga, C. M. (2021). Antimicrobial Activity of Chemically and Biologically Treated Chitosan Prepared from Black Soldier Fly (*Hermetia illucens*) Pupal Shell Waste. *Microorganisms*, *9*(12), 2417.

Leke-Aladekoba, A. A. (2018). Comparison of extraction methods and characterisation of chitin and chitosan with antimicrobial and antioxidant properties from black soldier fly (*Hermetia illucens*) meal.

Leni, G., Soetemans, L., Jacobs, J., Depraetere, S., Gianotten, N., Bastiaens, L., ... & Sforza, S. (2020). Protein hydrolysates from *Alphitobius diaperinus* and *Hermetia illucens* larvae treated with commercial proteases. *Journal of Insects as Food and Feed*, *6*(4), 393-404.

Li, H., Yang, Z., Fu, L., Yuan, Z., Gao, C., Sui, X., ... & Guo, Q. (2021). Advanced polymer-based drug delivery strategies for meniscal regeneration. *Tissue Engineering Part B: Reviews*, *27*(3), 266-293.

Li, J., & Zhuang, S. (2020). Antibacterial activity of chitosan and its derivatives and their interaction mechanism with bacteria: Current state and perspectives. *European Polymer Journal*, *138*, 109984.

Liu, X., Chen, X., Wang, H., Yang, Q., ur Rehman, K., Li, W., ... & Zheng, L. (2017). Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly. *PLoS One*, *12*(8), e0182601.

Lopez- Moya, F., Martin- Urdiroz, M., Oses- Ruiz, M., Were, V. M., Fricker, M. D., Littlejohn, G., ... & Talbot, N. J. (2021). Chitosan inhibits septin- mediated plant infection by the rice blast fungus *Magnaporthe oryzae* in a protein kinase C and Nox1 NADPH oxidase- dependent manner. *New Phytologist*, *230*(4), 1578-1593.

Lu, Y., Sun, Q., She, X., Xia, Y., Liu, Y., Li, J., & Yang, D. (2013). Fabrication and characterisation of α-chitin nanofibers and highly transparent chitin films by pulsed ultrasonication. *Carbohydrate polymers*, *98*(2), 1497-1504.

Ma, Z., Garrido-Maestu, A., & Jeong, K. C. (2017). Application, mode of action, and in vivo activity of chitosan and its micro-and nanoparticles as antimicrobial agents: A review. *Carbohydrate polymers*, *176*, 257-265.

Maluin, F. N., & Hussein, M. Z. (2020). Chitosan-based agronanochemicals as a sustainable alternative in crop protection. *Molecules*, *25*(7), 1611.

Marei, N. H., Abd El-Samie, E., Salah, T., Saad, G. R., & Elwahy, A. H. (2016). Isolation and characterization of chitosan from different local insects in Egypt. *International journal of biological macromolecules*, *82*, 871-877.

Mati-Baouche, N., Elchinger, P.-H., de Baynast, H., Pierre, G., Delattre, C., & Michaud, P. (2014). Chitosan as an adhesive. *European Polymer Journal*, 60, 198–212.

Meng, D., Garba, B., Ren, Y., Yao, M., Xia, X., Li, M., & Wang, Y. (2020). Antifungal activity of chitosan against *Aspergillus ochraceus* and its possible mechanisms of action. *International journal of biological macromolecules*, *158*, 1063-1070.

Mehra, N. K., Mishra, V., & Jain, N. K. (2014). A review of ligand tethered surface engineered carbon nanotubes. *Biomaterials*, *35*(4), 1267-1283.

Mohamed, N., & Madian, N. G. (2020). Evaluation of the mechanical, physical and antimicrobial properties of chitosan thin films doped with greenly synthesized silver nanoparticles. *Materials Today Communications*, *25*, 101372.

Moussout, H., Ahlafi, H., Aazza, M., & Bourakhouadar, M. (2016). Kinetics and mechanism of the thermal degradation of biopolymers chitin and chitosan using thermogravimetric analysis. *Polymer Degradation and Stability*, 130, 1–9.

Nair, A., Mallya, R., Suvarna, V., Khan, T. A., Momin, M., & Omri, A. (2022). Nanoparticles—Attractive Carriers of Antimicrobial Essential Oils. *Antibiotics*, *11*(1), 108.

Namangkalakul, W., Benjavongkulchai, S., Pochana, T., Promchai, A., Satitviboon, W., Howattanapanich, S., ... & Matangkasombut, O. (2020). Activity of chitosan antifungal denture adhesive against common Candida species and *Candida albicans* adherence on denture base acrylic resin. *The Journal of prosthetic dentistry*, *123*(1), 181-e1.

Ndueze, O. U., Okiwelu, S. N., Umeozor, O. C., & Noutcha, M. A. E. (2013). Arthropod succession on wildlife carcasses in lowland rainforest, Rivers State, Nigeria. *European Journal of Experimental Biology, 3(5), 106–110.*

Nechita, P. (2017). Applications of chitosan in wastewater treatment. *Biological activities and application of marine polysaccharides*, *1*, 209-228.

Ólafsdóttir, S. (2020). Comparison of Different Assay Methods and Sample Preparation Procedures for the Evaluation of the Antibacterial Activity of Chitosan (Doctoral dissertation).

Oonincx, D. G. A. B., & Finke, M. D. (2021). Nutritional value of insects and ways to manipulate their composition. *Journal of Insects as Food and Feed*, *7*(5), 639-659.

Pachapur, V. L., Guemiza, K., Rouissi, T., Sarma, S. J., & Brar, S. K. (2016). Novel biological and chemical methods of chitin extraction from crustacean waste using saline water. *Journal of Chemical Technology & Biotechnology*, *91*(8), 2331-2339.

Paul, T., Halder, S. K., Das, A., Ghosh, K., Mandal, A., Payra, P., ... & Mondal, K. C. (2015). Production of chitin and bioactive materials from Black tiger shrimp (*Penaeus monodon*) shell waste by the treatment of bacterial protease cocktail. *3 Biotech*, *5*(4), 483-493.

Perinelli, D. R., Fagioli, L., Campana, R., Lam, J. K., Baffone, W., Palmieri, G. F., ... & Bonacucina, G. (2018). Chitosan-based nanosystems and their exploited antimicrobial activity. *European Journal of Pharmaceutical Sciences*, *117*, 8-20.

Philibert, T., Lee, B. H., & Fabien, N. (2017). Current status and new perspectives on chitin and chitosan as functional biopolymers. *Applied Biochemistry and Biotechnology*, 181(4), 1314–1337.

Riaz Rajoka, M. S., Mehwish, H. M., Wu, Y., Zhao, L., Arfat, Y., Majeed, K., & Anwaar, S. (2020). Chitin/chitosan derivatives and their interactions with microorganisms: a comprehensive review and future perspectives. *Critical reviews in biotechnology*, *40*(3), 365-379.

Sargin, I., Arslan, G., & Kaya, M. (2019). Production of magnetic chitinous microcages from ephippia of zooplankton *Daphnia longispina* and heavy metal removal studies. *Carbohydrate polymers*, *207*, 200-210.

Shapi'i, R. A., Othman, S. H., Nordin, N., Basha, R. K., & Naim, M. N. (2020). Antimicrobial properties of starch films incorporated with chitosan nanoparticles: In vitro and in vivo evaluation. *Carbohydrate polymers*, *230*, 115602.

Shariatinia, Z. (2020). Biopolymeric nanocomposites in drug delivery. In *Advanced Biopolymeric Systems for Drug Delivery* (pp. 233-290). Springer, Cham.

Shumo, M., Osuga, I. M., Khamis, F. M., Tanga, C. M., Fiaboe, K. K., Subramanian, S., ... & Borgemeister, C. (2019). The nutritive value of black soldier fly larvae reared on common organic waste streams in Kenya. *Scientific reports*, *9*(1), 1-13.

Silva, C., Matamá, T., Kim, S., Padrão, J., Prasetyo, E. N., Kudanga, T., ... & Cavaco-Paulo, A. (2020). Antimicrobial and antioxidant linen via laccase-assisted grafting. *Reactive and Functional Polymers*, *71*(7), 713-720.

Spiridon, I., Andrei, I. M., Anghel, N., Dinu, M. V., & Ciubotaru, B. I. (2021). Development and Characterization of Novel Cellulose Composites Obtained in 1-Ethyl-3-methylimidazolium Chloride Used as Drug Delivery Systems. *Polymers*, *13*(13), 2176.

Spranghers, T., Ottoboni, M., Klootwijk, C., Ovyn, A., Deboosere, S., De Meulenaer, B., ... & De Smet, S. (2017). Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. *Journal of the Science of Food and Agriculture*, *97*(8), 2594-2600.

Tareq, A., Alam, M., Raza, S., Sarwar, T., Fardous, Z., Chowdhury, A. Z., & Hossain, S. (2013). Comparative study of antibacterial activity of chitin and chemically treated chitosan prepared from shrimp (*Macrobrachium Rosenbergii*) shell waste. *J Virol Microbiol*, *9*.

Tourón, N., El Khalfi, B., Yassine, S. M., Chabir, R., Errachidi, F., & Soukri, A. (2016). Rheological and antioxidant characterization of chitin and chitosan extracted with different acids. *Journal of Chitin and Chitosan Science*, *4*(1), 41-45.

Tsurkan, M. V., Voronkina, A., Khrunyk, Y., Wysokowski, M., Petrenko, I., & Ehrlich, H. (2020). Progress in chitin analytics. *Carbohydrate Polymers*, 117204.

Umoren, S. A., Solomon, M. M., Nzila, A., & Obot, I. B. (2020). Preparation of Silver/Chitosan Nanofluids Using Selected Plant Extracts: Characterization and Antimicrobial Studies against Gram-Positive and Gram-Negative Bacteria. *Materials*, *13*(7), 1629.

Usman, A., Zia, K. M., Zuber, M., Tabasum, S., Rehman, S., & Zia, F. (2016). Chitin and chitosan-based polyurethanes: A review of recent advances and prospective biomedical applications. *International journal of biological macromolecules*, *86*, 630-645.

Vandeweyer, D., Bruno, D., De Smet, J., Bonelli, M., Casartelli, M., Tettamanti, G., & Van Campenhout, L. (2020). Black soldier fly larvae rearing on side streams: impact on bacterial biota in different gut regions and larval growth. In *Insects to feed the world Virtual Conference: November, 23rd-26th* (Vol. 6, No. Supplement 1). Wageningen Academic Publishers.

Verlee, A., Mincke, S., & Stevens, C. V. (2017). Recent developments in antibacterial and antifungal chitosan and its derivatives. *Carbohydrate polymers*, *164*, 268-283.

Verma, M. L., Kumar, S., Das, A., Randhawa, J. S., & Chamundeeswari, M. (2020). Chitin and chitosan-based support materials for enzyme immobilization and biotechnological applications. *Environmental Chemistry Letters*, *18*(2), 315-323.

Victor, R., Marcelo da Cunha Santos, A., Viana de Sousa, B., de Araújo Neves, G., Navarro de Lima Santana, L., & Rodrigues Menezes, R. (2020). A review on Chitosan's uses as biomaterial: tissue engineering, drug delivery systems and cancer treatment. *Materials*, *13*(21), 4995.

Vogel, H., MŘller, A., Heckel, D. G., Gutzeit, H., & Vilcinskas, A. (2018). Nutritional immunology: diversification and diet-dependent expression of antimicrobial peptides in the black soldier fly *Hermetia illucens*. *Developmental & Comparative Immunology*, *78*, 141-148.

Wang, H., ur Rehman, K., Feng, W., Yang, D., ur Rehman, R., Cai, M., ... & Zheng, L. (2020). Physicochemical structure of chitin in the developing stages of black soldier fly. *International journal of biological macromolecules*, *149*, 901-907.

Wang, Shelomi M. (2017) Review of black soldier fly (*Hermetia illucens*) as animal feed and human food. *Foods*. 2017;6(10):91.

Wanule, D., Balkhande, J. V., Ratnakar, P. U., Kulkarni, A. N., & Bhowate, C. S. (2014). Extraction and FTIR analysis of chitosan from American cockroach, *Periplaneta americana*. *3*(3), 299-304.

Wong, C. Y., Rosli, S. S., Uemura, Y., Ho, Y. C., Leejeerajumnean, A., Kiatkittipong, W., ... & Lim, J. W. (2019). Potential protein and biodiesel sources from black soldier fly larvae: insights of larval harvesting instar and fermented feeding medium. *Energies*, *12*(8), 1570.

Xia, G., Zhai, D., Sun, Y., Hou, L., Guo, X., Wang, L., ... & Wang, F. (2020). Preparation of a novel asymmetric wettable chitosan-based sponge and its role in promoting chronic wound healing. *Carbohydrate polymers*, *227*, 115296.

Xing, K., Liu, Y., Shen, X., Zhu, X., Li, X., Miao, X., ... & Qin, S. (2017). Effect of O-chitosan nanoparticles on the development and membrane permeability of Verticillium dahliae. *Carbohydrate polymers*, *165*, 334-343.

Yeul, V. S., & Rayalu, S. S. (2013). Unprecedented chitin and chitosan: a chemical overview. *Journal of Polymers and the Environment*, 21(2), 606–614.

Younes, I., & Rinaudo, M. (2015). Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Marine drugs*, *13*(3), 1133-1174.

Youssef, K., & Hashim, A. F. (2020). Inhibitory Effect of Clay/Chitosan Nanocomposite against *Penicillium digitatum* on Citrus and Its Possible Mode of Action. *Jordan Journal of Biological Sciences*, *13*(3).

Yuan, Y., Chen, L., Shi, W., Wang, Z., & Zhang, H. (2020). Insight into physicochemical, rheological, and antibacterial properties of chitosan extracted from antarctic krill: A comparative study. *Molecules*, *25*(18), 4074.

Zaku, S. G., Aguzue, S., & Thomas, S. A. (2011). Extraction and characterization of chitin ; a functional biopolymer obtained from scales of common carp fish ( *Cyprinus Carpio* ): A lesser-known source. *African Journal of Food Science*, 5(8), 478–483.

Zare, E. N., Makvandi, P., & Tay, F. R. (2019). Recent progress in the industrial and biomedical applications of tragacanth gum: A review. *Carbohydrate Polymers*, *212*, 450-467.

Zhang, Y., Chwee, T. L., Ramakrishna, S., & Huang, Z. M. (2014). Recent development of polymer nanofibers for biomedical and biotechnological applications. *Journal of Materials Science*, 16(10), 933–946.

Zheng, Y., Pan, N., Liu, Y., & Ren, X. (2021). Novel porous chitosan/N-halamine structure with efficient antibacterial and hemostatic properties. *Carbohydrate Polymers*, *253*, 117205.

Zhou, G., Zhang, H., He, Y., & He, L. (2010). Identification of a chitin deacetylase producing bacteria isolated from soil and its fermentation optimization. *African Journal of Microbiology Research*, 4(23), 2597–2603.

Zhou, Z., Jiang, F., Lee, T. C., & Yue, T. (2013). Two-step preparation of nano-scaled magnetic chitosan particles using Triton X-100 reversed-phase water-in-oil microemulsion system. *Journal of Alloys and Compounds*, 581, 843–848.

Zimri, M. N. (2018). Preparation and electrospinning of chitosan from waste black soldier fly biomass.

Złotko, K., Waśko, A., Kamiński, D. M., Budziak-Wieczorek, I., Bulak, P., & Bieganowski, A. (2021). Isolation of Chitin from Black Soldier Fly (*Hermetia illucens*) and Its Usage to Metal Sorption. *Polymers*, *13*(5), 818.

## APPENDICES

**Appendix I: ANOVA of yield of chitin obtained from BSF exuviae using chemical and biological methods**

| Source of Variation | SS | Df | MS | F | Prob>F | F-crit |
|---|---|---|---|---|---|---|
| Between groups | 30.503 | 5 | 6.101 | 5.080 | 0.009 | 2.410 |
| Within groups | 14.413 | 12 | 1.201 | | | |
| Total | 44.916 | 17 | | | | |

**Appendix II: ANOVA of antimicrobial properties of BSF-derived chitosan against selected pathogenic microbes**

| Source of Variation | SS | Df | MS | F | Prob>F | F-crit |
|---|---|---|---|---|---|---|
| Between groups | 927.117 | 3 | 309.039 | 36.710 | 0.00001 | 3.240 |
| Within groups | 471.467 | 56 | 8.419 | | | |
| Total | 1398.584 | 59 | | | | |

**Appendix III: ANOVA table for the comparative effects of antimicrobial activities of BSF chitosan, shrimp chitosan and convectional antibiotics against *C. albicans***

| Source of Variation | SS | Df | MS | F | Prob>F | F-crit |
|---|---|---|---|---|---|---|
| Between groups | 1959.200 | 9 | 217.690 | 82.670 | 0.001 | 2.630 |
| Within groups | 20.000 | 52.7 | | | | |
| Totals | 1979.200 | 58.7 | | | | |

**Appendix IV: ANOVA table for the comparative effects of antimicrobial activities of BSF chitosan, shrimp chitosan and convectional antibiotics against *E. coli***

| Source of Variation | SS | Df | MS | F | Prob>F | F-crit |
|---|---|---|---|---|---|---|
| Between groups | 1977.000 | 9 | 219.660 | 74.040 | 0.001 | 2.970 |
| Within groups | 20.000 | 59 | | | | |
| Total | 1997.000 | 68 | | | | |

**Appendix V: ANOVA table for the comparative effects of antimicrobial activities of BSF chitosan, shrimp chitosan and convectional antibiotics against *S. aureus***

| Source of Variation | SS | Df | MS | F | Prob>F | F-crit |
|---|---|---|---|---|---|---|
| Between groups | 3171 | 9 | 352.300 | 81.930 | 0.001 | 4.300 |
| Within groups | 20 | 86 | | | | |
| Total | 3191 | 95 | | | | |

**Appendix VI: ANOVA table for the comparative effects of antimicrobial activities of BSF chitosan, shrimp chitosan and convectional antibiotics against *B. subtilis***

| Source of Variation | SS | Df | MS | F | Prob>F | F-crit |
|---|---|---|---|---|---|---|
| Between groups | 4324 | 9 | 480.400 | 42 | 0.001 | 1.100 |
| Within groups | 20 | 23 | | | | |
| Total | 4344 | 32 | | | | |

**Appendix VII: ANOVA table for the comparative effects of antimicrobial activities of BSF chitosan, shrimp chitosan and convectional antibiotics against *P. aeruginosa***

| Source of Variation | SS | Df | MS | F | Prob>F | F-crit |
|---|---|---|---|---|---|---|
| Between groups | 3305 | 9 | 367.300 | 25 | 0.001 | 1.400 |
| Within groups | 20 | 29 | | | | |
| Total | 3325 | 38 | | | | |