AMJ
ISSN: 0005-2523

ISSN: 0005-2523
Volume 63, Issue 06, June, 2023

Potention Black Soldier Fly's (Hermetia illucens) Live for Wound Healing and Bone Remodeling: A Systematic Review

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Keywords:
Bone Remodeling, Black Soldier Fly (BSF), Chitosan, Wound Healing.

ABSTRACT

The Black Soldier Fly (BSF) (Hermetia illucens) is included in the decomposer fly which is not a disease vector. BSF is widely cultivated because it has high nutritional value with a protein content of 44.26% and a fat content of 29.65%. BSF life has the potential as a raw material in the chitosan manufacturing process because it contains the chemical compounds chitin and chitosan. Chitosan is able to accumulate collagen fibers and produce faster wound healing and as an alternative material for bone remodeling. To find out the potential of BSF life as wound healing and bone remodeling. Database obtained from PubMed, Science direct, Google Schooler databases for the last 5 years from 2019-2023. The keyword used in this research is BSF. The study selection process was included in the data meta-analysis using PRISMA-2020. Screening of full text articles was included in a systematic review based on inclusion and exclusion criteria. The search resulted in a total of 18 articles being entered based on the eligibility criteria. The study search categories obtained include BSF characteristics including larvae (maggot), pupae and shells as wound healing and bone remodeling materials, information related to chitosan from BSF, namely the method of making chitosan, characteristics of chitosan test results, the benefits of chitosan in the process of wound healing and bone remodeling. The purity of BSF chitosan is proven through the FTIR, XRD and TGA functional groups which are recognized by FDA (Florida Dental Association). Chitosan is a bioactive material in bone tissue engineering which can be processed in the form of a gel or scaffold. Chitosan improves osteogenic performance so that it can be used as a material for the treatment of bone defects by increasing the expression of Osteporotegerin (OPG), osteocalcin, BMP-2, osteoblasts and decreasing proinflammatory cytokines IL-6, TNF-α, RANKL which have all achieved good therapeutic effects.

1. Introduction

The process of wound healing is a complex biological process involving sequential biochemical and cellular responses, in general the process is divided into five phases consisting of the inflammatory phase which appears immediately after the lesion, the migratory phase, the proliferative phase (including fibroplasia, neovascularization, the formation of granulation tissue, reepithelialization), and maturation, which ultimately results in a remodeling. Through advances in scientific technology, various materials are used from both natural and synthetic sources to be used in wound healing processes. Organic and synthetic materials used as wound dressings function to maintain moisture, electrolyte balance, hemostatic, analgesic, healing, and antimicrobial properties [1], [2].

The use of Black Soldier Fly (BSF) in wound care (larval therapy) has been around for several centuries, namely by placing thousands of BSF larvae in chronic wounds so that the BSF larvae only attack necrotic tissue. BSF larvae therapy can trigger the formation of granulation tissue thereby triggering the healing of infected wounds which was later proposed by several surgeons in France in the 16th and 18-19th centuries. BSF is not a vector of pathogens and is not dangerous to human health [3], [4].

During the prepupa to pupa phase, BSF can be used as a potential source of chitin because the exoskeleton of the BSF contains 35% chitin which is found in shedding and cocoons. BSF (Hermetia illucens) has a fairly high protein content of around 40-50%. BSF pupa shell contains chitin which can be converted into chitosan. Chitosan has biodegradable and biocompatible properties, antibacterial, antimicrobial, antifungal, analgesic, antitumor, high bioavailability, good water permeselectivity, and high chemical resistance and non-toxicity. Chitosan has a high binding capacity so that it can be applied as an absorbent and drug delivery medium composed of amino-NH2 groups and –OH hydroxy groups [5], [6].

Chitosan is an amino polysaccharidae with the chemical formula N-acetyl-D-glucosamine whose polymer structure is the same as hyaluronic acid, namely the glycosaminoglycan group (GAGs), which are extracellular matrix macromolecules that are important for wound healing. Chitosan will gradually depolymerize to release Naacetyl-β-D-glucosamine, which stimulates fibroblast proliferation, HA synthesis, angiogenesis, and provides collagen deposits at the wound site. Chitosan is a natural polymer material derived from deacetylated chitin, has biocompatible properties and has high osteointegration and osteoconductive abilities to stimulate osteoblast formation and reduce osteoclast activity so that it is suitable for use as bone regeneration. Chitosan is a biomaterial that has been used to accelerate the healing process wounds of extraction. Chitosan was able to increase transforming growth factor beta 1 (TGF-β1), platelet release transforming growth factor (PDGF), fibroblast growth factor-2 (FGF2), BMP mRNA expression on the seventh day. Chitosan has an effect as a blood and tissue interface in the healing process and is proven to be a biocompatible material in bone remodeling, as well as osteoconduction providing a physical effect as a graft in the formation of new bone. Osteoconduction causes the differentiation of mesenchymal cells into bone cells and stimulates bone remodeling [7- 9].

The benefits of chitosan in the health sector include, chitosan is used for bacteriostatic, immunology, antitumor, cicatrizant, homeostatic and anti-coagulant, ointment for wounds, ophthalmology, orthopedics, and healing sutures due to surgery. Chitosan is known to have biodegradability, biocompatibility and non-toxic properties, therefore, chitin and chitosan have received great attention in medical and pharmaceutical applications. In addition, chitosan biopolymers are considered as significant antimicrobial agents for wound healing, along with their hemostatic and analgesic functions [8].

2. Methods

2.1 Database Searching and Study Selection

A systematic literature search was conducted to identify articles about part of the Black Soldier Fly in helping accelerate the process of wound healing and bone remodeling after tooth extraction. To prevent selection bias, the predetermined inclusion criteria used were all literature discussing the utilization of black soldier fly starting from larvae/maggot, pupae and shells which were processed into chitosan for wound healing and bone remodeling, database searches took place in the last 5 year period from 2019 -2023 retrieved from PubMed, Science direct, Google Schooler database searched systematically to identify appropriate studies. The reference list was also screened for additional studies. Exclusion criteria for research studies that are not journal articles, information on the development of the methodology is insufficient.

Titles and abstracts are filtered to exclude irrelevant articles. Screening of full text articles was then performed to identify studies to be included in the systematic review based on inclusion and exclusion criteria. Identify reporting using PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses).

2.2 Search strategy

Data was extracted based on three categories: 1) The characteristics of the black soldier fly including larvae or maggot, pupae and shells as wound healing and bone remodeling materials. 2) Information related to chitosan from the black soldier fly, namely the method of making chitosan, the characteristics of the test results for chitosan, the benefits of chitosan in the process of wound healing and bone remodeling, 3) The list of references from systematic reviews and other existing ones is also explored for other relevant articles.

The search keywords are "black soldier fly, chitosan, wound healing, bone remodeling." A comprehensive search strategy was developed and conducted to find relevant articles. Search strategy for PubMed: “maggot black soldier fly”, “larva black soldier fly”, “pupa black soldier fly”, “shell black soldier fly”. Chitosan (all fields), "chitosan and wound healing" (all fields), "chitosan and bone remodeling" (all fields), "chitosan pupa black soldier fly", "chitosan black soldier fly shell", "wound healing" (all fields), “bone remodeling” (all fields), “potential of chitosan” (all fields), “potential of black soldier fly”, “consumption of black soldier fly for humans”, “benefits of black soldier fly for health” (all fields). Search strategy for Google schooler: "black soldier fly effect", "use of insects for health", potential of chitosan insects” (all fields), “black soldier fly as a food alternative”, “wound healing using chitosan” (all fields), “bone remodeling using chitosan” (all fields), “chitosan black soldier fly” (all fields). Search strategy for Science direct: "Insect chitosan", "benefits of chitosan" (all fields), "chitosan as wound healing", "chitosan as bone remodeling", "black soldier fly protein", "protein and wound healing" (all fields), “protein and bone remodeling” (all fields). Reference lists from existing systematic and other reviews were also explored for other relevant and overlooked articles. Search strategy for Science direct: "Insect chitosan", "benefits of chitosan" (all fields), "chitosan as wound healing", "chitosan as bone remodeling", "black soldier fly protein", "protein and wound healing" (all fields), “protein and bone remodeling” (all fields). Reference lists from existing systematic and other reviews were also explored for other relevant and overlooked articles. Search strategy for Science direct: "Insect chitosan", "benefits of chitosan" (all fields), "chitosan as wound healing", "chitosan as bone remodeling", "black soldier fly protein", "protein and wound healing" (all fields), “protein and bone remodeling” (all fields). Reference lists from existing systematic and other reviews were also explored for other relevant and overlooked articles.

3. Results

A total of 18 most relevant articles were included in the systematic objective. The selection process for studies to be included in the data meta-analysis using PRISMA-2020 is shown in Figure 1. The main reasons for the exclusion of articles were different results, study designs, research methods, chitosan base material which was not used for wound healing and bone remodeling, not chitosan material in the process of wound healing and bone remodeling. Table 1 shows a summary of the data extracted from all the studies included in this review. The data table consists of the author, the year of publication of the journal, the title of the research journal, the research method and research results and conclusions.

3.1 Making chitosan from BSF life

Cultivation of the Black Soldier Fly (Hermetia illucens) is increasingly being developed throughout the world. This is because the pupa phase of BSF is known to have a high protein content (up to 40%) so that it is widely cultivated as an alternative protein raw material. The chitosan extraction process from either the pupa or the BSF pupa shell consists of 4 stages, namely demineralization, deproteination, depigmentation, and deacetylation. The results of the characterization of chitosan products obtained, both at variations in temperature and extraction time, have a white color that tends to be yellowish with a fine powder texture. Some studies on the manufacture of chitosan from pupa shells from BSF concluded that the results of the isolation of chitosan from BSF pupa shells based on organoleptic tests showed that chitosan from BSF pupa shells met the SNI chitosan quality standard parameters No. 7949-2013 is white-light brown in color, in the form of flakes to fine powder. Based on functional group analysis using FT-IR, BSF chitosan pupae can be seen for an absorption band in the region of wave number 3274.58 cm-1 which indicates the presence of hydroxyl groups which are absorbed in the area 1638.72 cm-1 which indicates the presence of amide groups.

FTIR spectra analysis of isolated chitosan showed that the formation of chitosan was indicated by the appearance of O-H, NH and C=O vibrations. The higher the deacetylation temperature, the faster the reaction of converting chitin into chitosan, due to the weakening of intermolecular bonds due to heat which causes faster intermolecular motion. The –OH group from NaOH is added to chitin, thus eliminating the acetyl group to produce a free amine which will bind to become an amide. The degree of deacetylation (DD) is a parameter that measures the number of acetyl groups in the loose chitin. The standard degree of deacetylation of chitosan is 70-80%. The greater the DD value, the better the quality of the chitosan produced. The resulting chitosan will be more reactive because more amine groups replace the acetyl groups (amine groups are more reactive than acetyl groups due to the presence of PEB on the nitrogen atom in the chitosan structure) [5].

3.2 The Role of Chitosan in Wound Healing

Various extensive searches in study reports included that the benefits of chitosan in the health sector include chitosan can be used for bacteriostatic, immunology, antitumor, cicatrizant, homeostatic and anti-coagulant, ointment for wounds, ophthalmology, orthopedics, and healing sutures due to surgery. Chitosan has attractive properties that make it suitable for use in biomedical applications. Chitosan is known to have biodegradability, biocompatibility and non-toxic properties, therefore, chitin and chitosan have received great attention in medical and pharmaceutical applications. In addition, chitosan biopolymers are considered as significant antimicrobial agents for wound healing, along with their hemostatic and analgesic functions [2], [4], [5].

ISSN: 0005-2523
Volume 63, Issue 06, June, 2023

Most of the biological properties of chitosan are related to its cationic activity and the size of its polymer chains. These characteristics make chitosan ideal for use as a wound dressing. The main biochemical activities of chitin and chitosan-based materials in the wound healing process are through polymorphonuclear cell (PMN) activation, fibroblast activation, cytokine production, giant cell migration, and stimulation of type IV collagen synthesis. Chitosan biopolymer effectively depolymerizes to release N-acetyl-β-D-glucosamine to initiate fibroblast proliferation during the wound healing process. Chitosan monomer also helps in regulating collagen deposition and stimulates increased levels of natural hyaluronic acid synthesis at the wound site. Chitosan also provides a cellulose matrix for skin tissue regeneration and activates macrophages to stop abnormal growth activity. This helps the wound healing process faster and prevent the appearance of scars [1], [3].

Chitosan has a positive charge from the deacetylation process of N-acetyl-D-glucosamine to form D-glucosamine so as to provide a free amino group in its molecular structure. The cationic nature of chitosan is used to induce hemostasis because the surfaces of platelets and erythrocytes show a hemostatic charge due to the presence of phosphatidylcholine, phosphatidylethanolamine and sialic acid groups. The amino group present in chitosan (poly-N-acetyl-D-glucosamine) is involved in facilitating erythrocyte aggregation through electrostatic interactions with its surface charge, and subsequently induced hemostasis after activating platelets. The molecular weight and degree of deacetylation achieved during the chitosan purification process will significantly affect the hemostatic ability of chitosan. A higher degree of deacetylation increases the aggregation of erythrocytes and platelets which is necessary to initiate hemostasis. Chitosan and its derivatives have shown antimicrobial activity against various microorganisms, such as bacteria, fungi and viruses. The antimicrobial activity of chitosan is highly dependent on its molecular weight and concentration. Chitosan with high molecular weight showed increased antimicrobial activity against gram-positive Staphylococcus aureus, while chitosan with low molecular weight showed high antimicrobial activity against gram-negative [1], [3- 6].

Many studies reveal that during the inflammatory phase, the application of chitosan to wounds will cause chitosan to be actively polymerized to secrete N-acetyl glucosamine by lysosomal enzymes, then the N-acetyl glucosamine monomer binds to the main receptor on macrophages and triggers the migration and proliferation of macrophage cells. The greater the number of macrophages in a wound tissue, the faster the wound healing process, especially during the inflammatory phase. Stimulation of macrophage cells using chitosan showed an increase in Transforming Growth Factor Beta 1 (TGF β1), Platelets Release Transforming Growth Factor (PDGF), VEGF and Fibroblast Growth Factor 2 (FGF2) to enter the proliferative phase. FGF2 plays an important role in the development of granulation tissue, fibroblast proliferation, epithelial cell proliferation and angiogenesis. Growth factors are also released by platelets and fibroblasts, including FGF-2 which is also produced by monocytes, keratinocytes and fibroblasts. This phase results in the development of tissue which will eventually be replaced by bone. When the tissue is resorbed, cells such as fibroblasts, chondroblasts, and osteoblasts will begin to form by pluripotent mesenchymal cells. Fibroblast tissue will stimulate OPG to inhibit the binding of RANKL to RANK and trigger the growth of FGF2. OPG will also express a transcription factor, Runx2, which is the earliest osteoblast marker and will affect mesenchymal cells (MSC) and osteoblast cells as well as modulate Runx2 expression to help osteoblasts form. This phase results in the development of tissue which will eventually be replaced by bone. When the tissue is resorbed, cells such as fibroblasts, chondroblasts, and osteoblasts will begin to form by pluripotent mesenchymal cells. Fibroblast tissue will stimulate OPG to inhibit the binding of RANKL to RANK and trigger the growth of FGF2. OPG will also express a transcription factor, Runx2, which is the earliest osteoblast marker and will affect mesenchymal cells (MSC) and osteoblast cells as well as modulate Runx2 expression to help osteoblasts form. This phase results in the development of tissue which will eventually be replaced by bone. When the tissue is resorbed, cells such as fibroblasts, chondroblasts, and osteoblasts will begin to form by pluripotent mesenchymal cells. Fibroblast tissue will stimulate OPG to inhibit the binding of RANKL to RANK and trigger the growth of FGF2. OPG will also express a transcription factor, Runx2, which is the earliest osteoblast marker and will affect mesenchymal cells (MSC) and osteoblast cells as well as modulate Runx2 expression to help osteoblasts form. Fibroblast tissue will stimulate OPG to inhibit the binding of RANKL to RANK and trigger the growth of FGF2. OPG will also express a transcription factor, Runx2, which is the earliest osteoblast marker and will affect mesenchymal cells (MSC) and osteoblast cells as well as modulate Runx2 expression to help osteoblasts form. Fibroblast tissue will stimulate OPG to inhibit the binding of RANKL to RANK and trigger the growth of FGF2. OPG will also express a transcription factor, Runx2, which is the earliest osteoblast marker and will affect mesenchymal cells (MSC) and osteoblast cells and modulation of Runx2 expression to help the formation of osteoblasts [12], [17- 30].

3.3 The Role of Chitosan in Bone Remodeling

Bone is a hard connective tissue and serves to form the framework of the human body. After injury, if the damage is minor and the size of the defect is small, bone tissue has a certain self-healing potential through osteogenic differentiation of bone marrow mesenchymal stem cells, bone neoformation and neo-angiogenesis at the lesion site. For large bone defects, bone grafts are usually required [32].

Scaffold is a biomaterial that facilitates more regular growth and formation of new bone tissue. In vitro and in vivo experiments showed that the chitosan scaffold is non-toxic, and has excellent biocompatibility, osteoconductivity and osteogenesis properties, promotes good cell proliferation and cell adhesion, causing the increase of new bone, regeneration. Regarding the more specific clinical application of chitosan in alveolar and jaw bone regeneration, several preclinical reports show promising results, even for accelerating osseointegration of dental implants and reconstructing critical size defects [32], [33].

Several studies using chitosan and other substances have shown beneficial effects on bone formation. Maryani found that the combination group*Platelet Rich Plasma*(PRP) and chitosan gel had significantly higher numbers of osteoblasts than the PRP, chitosan gel, and povidone iodine groups 14 days after tooth extraction in Wistar rats. This is also consistent with the findings of Danilchenko et al., who found bone tissue production due to increased osteoconductive characteristics in a mixture of hydroxyapatite and chitosan materials. Chitosan is a chitin derivative which can be obtained from various fishery products, including shellfish, crabs, shrimp and fish scales. In addition, chitin can also be produced from insects, one of which is the Black Soldier Fly (Hermetia illucens) [34- 36].

The RANKL, RANK, OPG system is known for its role in osteoclast maturation and bone remodeling. Receptor activator of NF-kB (RANK), receptor activator of NF-kB ligand (RANKL), and osteoprotegerin (OPG) are the main components. Djais's research on Utilization of Milkfish Chitosan (Chanos chanos) Scales as an alternative material for bone regeneration, showed that the group with the addition of chitosan showed a much higher OPG value than the negative control. The chitosan and chitosan+hydroxyapatite combination group showed higher OPG expression than the bone graft group. This suggests that the addition of chitosan can help increase the amount of OPG which helps the bone remodeling process. Chitosan is a natural polymer, biodegradable and biocompatible that has the potential to act as a scaffold that promotes bone healing [34].

Rahmitasari's study regarding the effect of chitosan combination on bone repair concluded that the combination of chitosan and collagen scaffold could enhance the healing process of bone defects in Wistar

ISSN: 0005-2523
Volume 63, Issue 06, June, 2023

rats by reducing RANKL expression. The Djais study obtained the appropriate results, namely a significant decrease in RANKL expression in the chitosan group and the chitosan + hydroxyapatite combination against the negative control group on the seventh day [6], [33].

Repair of bone defects depends on many factors, such as proliferation of bone progenitor cells and bone growth factor. In bone tissue engineering, bone substitutes play an important role in supporting cell adhesion, growth, and proliferation at the site of injury. As mentioned above, chitosan is similar to a component of the extracellular matrix (ECM) glycosaminoglycans, which creates a local microenvironment for cell growth and supports osteoblast proliferation, differentiation, and mineralization. Based on the explanation above, it can be underlined that chitosan has an effect on the process of accelerating bone remodeling [36].

3.4 Chitosan content in BSF

In the prepupa phase until they become pupae, BSF flies can be used as a potential source of chitin because the exoskeleton of the BSF flies contains as much as 35% chitin. The insect cuticle contains chitin which is in the matrix of proteins, lipids and other compounds. Wasko's research concluded that chitin extracted from BSF flies had distinctive physicochemical properties compared to previously discovered chitin. Chitin extracted from BSF flies had a low crystallinity index, which was 24.9% for adult flies and 35% for larvae. This indicates that chitin has the potential to be used as a biosorption material because chitin with a low crystallity index has a high absorption capacity. In addition, the levels of chitin in BSF also differ in each growth stage (larvae, prepupae, pupa, adult flies). Chitin from BSF maintenance was found to be a suitable source of chitin with a chitin content of 8% to 24%. Small differences in the physicochemical characteristics between the chitin samples at BSF were observed but they did not greatly affect further chitosan processing leading to the advantage that all the chitin-containing BSF fractions could be collected and processed into chitosan with the same degree of deacetylation (DD) [28], [38], [37].

Cell adhesion to chitosan depends on DD which is often used to express the number of amino groups in chitosan; only DD >60% indicated chitosan production from chitin. Chitosan is the only positively charged polysaccharide in nature, and its charge density depends on DD and pH values. DD also has an impact on the biocompatibility of chitosan. For example, higher DD increases amount of positive charges and interactions between chitosan and cells, there by increasing biocompatibility [32], [39].

Based on Kanto's research which concluded that chitin and chitosan has been successfully extracted from adult H. illucens flies. The randemen chitin obtained at 17,93 (w/w) of the total sample weight. Chitosan obtained from chitin deacetylation results have a degree of deacetylation of 74.74%. Morphology The surface of chitosan is rougher and denser than the surface morphology of chitin. Chitin is a natural polysaccharide which is considered as a biopolymer the most abundant. In various studies, chitin has been found and isolated from several organisms of crustaceans, arachnids, and insects. Wrong One insect that contains chitin in its shell is a fly *Hermetia illucens*. Chitin can be processed through a deacetylation process to produce chitosan [40].

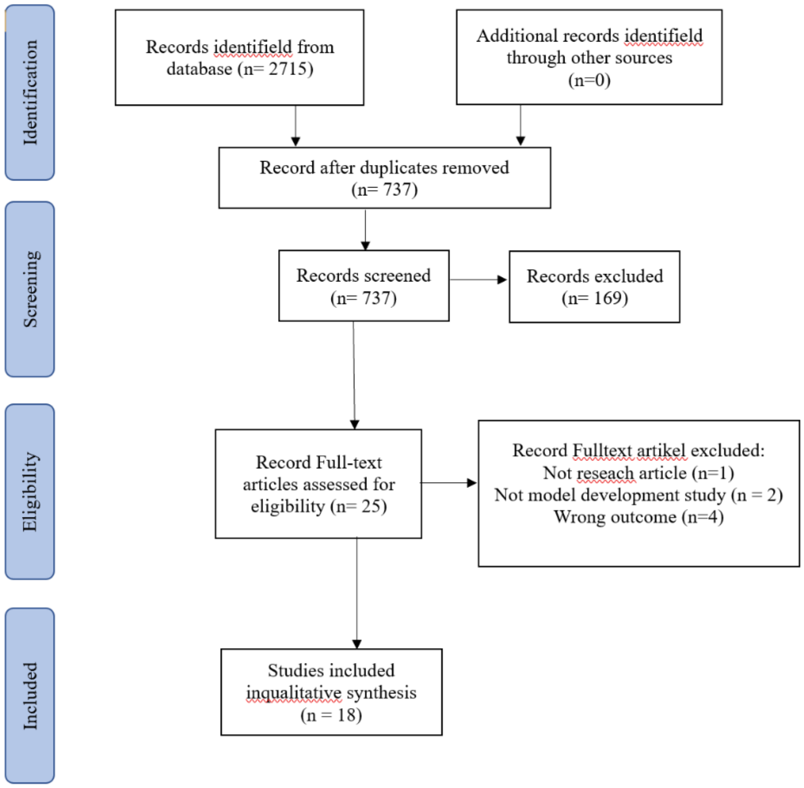

Figure 1. Prism diagram of research identification.

Table 1. Study reports from research on black soldier fly, chitosan, wound healing and bone healing

No	Writer	Article Title	Results	Conclusion
1.	[15]	Effectiveness of Combination of Chitosan Gel and Hydroxyapatite from Crabs Shells (Portunus pelagicus) Waste as Bonegraft on Periodontal Tissue Regeneration through IL-1 and BMP-2 Analysis	On days 7, 14 and 21, the expression of IL-1 in the chitosan and hydroxyapatite gel groups was lower than that of the positive control group, namely hydroxyapatite Batan (HA) and the negative control, namely placebo gel.	The addition of a combination of chitosan gel and hydroxyapatite can reduce IL-1 which is a pro-inflammatory cytokine, so that this compound can prevent inflammation and can increase BMP-2. When a bone injury occurs, BMP-2 production increases in week 2. BMP-2 will increase the processes of osteogenesis and osteoinduction. The formed osteoblasts will stimulate the formation of intercellular material, namely type I collagen, Alcaline phosphatase (ALP) and osteocalcin (OSN).
2.	Showkeen M, et all. 2022	Chitosan Nanoparticles: A Versatile Platform for Biomedical Applications	The different properties of chitosan make it an excellent biomaterial for various biomedical applications. One of the most prominent properties of chitosan	The use of nanochitosan particles is recognized by the FDA as safe for consumption as a drug and can be used as a tissue engineering system. In addition, nanochitosan

			is that it stimulates the immune system and does not cause inflammation. Chitosan with various degrees of deacetylation and molecular weight has low toxicity. Significantly, chitosan is a material that can be processed in various ways to produce a three-dimensional scaffold with a porous structure or in the form of nanoparticles which are used in bone tissue engineering and wound healing.	particles can increase the bioavailability of drugs and have low toxicity.
3.	[17]	The Validity of Nano-Chitosan/Nano-Hydroxyapatite as A Promoter of Bone Healing in Ovariectomized Rats	Decreased estrogen (hypoestrogenism) can lead to increased osteoclast activation, because RANK will bond with RANKL, estrogen plays a role in inhibiting RANK binding with RANKL. Estrogen also has a function in increasing osteoprotegerin (OPG). The combination of nano-chitosan and nano-hydroxyapatite can help bone repair even in female rats that have undergone ovariectomy. This happens because the combination of nano-chitosan and nano-hydroxyapatite can stimulate MSC cells to produce osteoblasts by increasing several growth factors. The combination of nano-chitosan and nano-hydroxyapatite also has osteoinduction and osteogenesis properties.	Estrogen deficiency can inhibit bone healing, but giving a combination of nano-chitosan and nano-hydroxyapatite can help improve bone healing even in conditions of estrogen deficiency. The combination of nano-chitosan and nano-hydroxyapatite is an active biomaterial for reducing impaired bone healing in rats with OVX-induced osteoporosis.
4.	Rostiny, et all. 2014.	Spirulina chitosan gel induction on the healing process of Cavia Cobaya post extraction socket	Induction of chitosan gel decreased the number of osteoclasts, increased the number of osteoblasts and collagen compared to	Chitosan gel is able to accumulate collagen fibers and produce faster wound healing and is an alternative material for bone remodeling after tooth

			the untreated control group.	extraction.
5.	Koutsos, et all. 2022	Immunomodulatory potential of black soldier fly larvae: applications beyond nutrition in animal feeding programs	Chitin and chitosan are also value added components of materials derived from BSF. The chitin content of BSFL (Black soldier fly larva) has been shown in larvae (3.6%), prepupae (3.1%), adults (2.9%), and pupal shells (14.1%). The content and form of chitin varies with larval age, due to changes in total concentration and degree of crystallization.	BSF is a type of insect that can be commercialized until now because it has chitin and chitosan which can be used as added value in the manufacture of a material. BSF oil and soybean oil can reduce levels of IL-6 and TNF-α.
6.	[3]	Effect of Maggot (H. Illucens) Methanol Extract on Open Wound Healing in Rats (Rattus Novergicus)	The effect of methanol maggot extract (EMM) (H.illucens) at concentrations of 10%, 15% and 20% was able to have an effect on healing open wounds in male white rats. The higher the concentration used, the greater the healing effect of open wounds.	The chitin content found in maggot (H. illucens) can accelerate blood clotting and the formation of fibrin threads thereby accelerating wound healing. The high protein content in maggot (H. illucens) in the form of ariginin, alanine, cysteine, glycine, proline and the minerals zinc (Zn) and copper (Cu) can accelerate wound healing through oral, parenteral and topical.
7.	Sivia N., et all. 2022	Extraction of Chitosan and Characterization of Nanoparticle Chitosan from Pupae Sheels of Black Soldier Fly (Hermetia Illucens)	Analysis of the infra-red chitosan spectra of isolated BSF pupae showed a distinctive functional group in chitosan, the yield of chitosan was 11.93%. Characterization of chitosan in the form of powder flakes, brownish white, odorless, moisture content 6.683%, ash content 0.33%, nitrogen content 4.091%, degree of deacetylation 94.41% so that it complies with SNI No.7949-2013.	Characterization of BSF Pupae nanoparticles formed with an average size (PSA) of 495.7 ± 41.54 nm, PDI (Polydispersity index) 0.551 ± 0.048 indicating that the nanoparticle formula is nanometer in size with a homogeneous/uniform size distribution.
8.	[20]	Bone remodeling using a	The first three days	Giving chitosan scaffold is

		three-dimensional chitosan - hydroxyapatite scaffold seeded with hypoxic conditioned human amnion mesenchymal stem cells.	after administration of the chitosan-HA scaffold, an inflammatory process occurred which was the initial phase of bone healing . During this phase, a hypoxic condition is created by the scaffold triggers an increase in VEGF expression which will induce the process of angiogenesis. Angiogenesis plays an important role in the bone healing process, with the formation of blood vessels will provide nutrients, oxygen Functional capillary network provides nutrition at the wound site. In the early stages of regeneration, MSC proliferation occurs followed by differentiation from osteoblasts. In the process of regeneration played by BMP2 proteins. At the next stage, activation the BMP2-triggered transcription factor RunX-2 helps induce MSC differentiation into osteoprogenitor preosteoblasts. The degree of bone matrix maturation is indicated by expression of type 1 collagen fibers. Deep mineralization the bone matrix maturation process will be affected by collagen type 1. If bone maturity increases there is also an increase in collagen type 1. The sign indicating that the osteoblasts are maturing is	superior in healing wounds in terms of osteogenic and angiogenic

			characterized by the presence of osteocalcin.	
9.	[21]	Bioactive 3D-printed chitosan-based scaffolds for personalized craniofacial bone tissue engineering.	chitosan and 5% HA were the best group in carrying out the bone healing process. As graft materials, chitosan and HA scaffolds have good biocompatibility because they can maintain normal cell immunity. No immediate or prolonged effect on host cells. The materials used in this study can accelerate the healing process of bone wounds because they have osteoconductive properties, increase bone cell adhesion, proliferation of the extracellular matrix.	The group that was the best at healing bones was the chitosan and HA 5% group.
10.	[22]	The Effect of White Shrimp Head Chitosan Gel (Litopenaeus vannamei) on Inhibitory Strength of Periodontopathogenic Bacteria and Accelerating Wound Healing (In Vitro, Histological, and Clinical Tests).	Chitosan has antibacterial properties, this is because chitosan has a positively charged amine group which can damage the bonds of the negatively charged bacterial cell wall. This situation can damage the permeability of the bacterial cell wall and cause an imbalance of pressure in the bacterial cell. Chitosan also has antibacterial properties due to the presence of lysozyme and aminopolysaccharide enzymes.	Chitosan gel can inhibit the growth of Aggregatibacter actinomycetemcomitans bacteria and accelerate the wound healing process.
11.	[23]	Analysis on Efficacy of Chitosan-based Gel on Bone Quality and Quantity.	The use of chitosan has several advantages, namely its biocompatibility, biodegrability, and antibacterial properties. Hydrogel formation in chitosan can form chitosan which is resistant to several stimuli, namely temperature, heat, pH, strong ionization, light	The chitosan-osteoprogerin gel group was better than the other groups in healing bones.

			etc. This study stated that the combination of chitosan-Opg gel was better at healing bone wounds. This is because the anion compounds of glycosaminoglycans from chitosan can increase several cytokines and growth factors. The advantage of the chitosan-Opg combination is that it can increase bone regeneration, besides that the chitosan compound in the hydrogel can also increase osteoblast proliferation thereby increasing bone matrix mineralization.	
12.	[24]	Improved treatment efficacy of risedronate functionalized chitosan nanoparticles in osteoporosis: formulation development, in vivo, and molecular modeling studies	Ovariectomy can cause a decrease in bone density, this is related to a decrease in estrogen function. Estrogen has a function to increase Osteoprogerin and inhibit osteoclast activation. At the time of ovariectomy there was a decrease in estrogen, and a decrease in osteoprogerin and an increase in osteoclasts. Bone density in the group that was given chitosan 1000 mg + risedronate sodium 500 mg showed bone density results that were similar to the group that did not have ovariectomy. This happens because the combination of chitosan and risedronate sodium can suppress bone resorption by inducing osteoclast apoptosis and increasing bone mass.	Chitosan group 1000 mg + risedronate sodium 500 can prevent bone damage.
13.	[25]	The effect of nano-hydroxyapatite/chitosan	The group that was given nano-chitosan	Nanochitosan can help the healing process of bone

		scaffolds on rat calvarial defects for bone regeneration.	was better at healing bones than the negative control group. Biologically, nano-chitosan can increase local inflammatory reactions which can increase angiogenesis and osteogenesis. The size of the nanochitosan used in this study is 20-40 nm in diameter and 80-160 nm in length.	wounds.
14.	[26]	Effect of chitosan scaffold on bone healing in rabbit calvarial defects.	This study observed 16 male rabbits which were divided into 2 groups, the first using autogenous and the second using chitosan scaffold with 3D shape. This study states that the use of autogenous is superior to 3D chitosan scaffold. The histological analysis showed that the use of 3D chitosan scaffold continued to improve bone healing.	This study concluded that although autogenous is superior to chitosan scaffolds formed in 3D, 3D chitosan scaffolds can also trigger bone growth, have good biocompatibility and are osteoconductive.
15.	[27]	Scaffold combination of chitosan and collagen synthesized from chicken feet induces osteoblast and osteoprotegerin expression in the bone healing process of mice.	research with 3 groups, namely the first using chitosan, the second using collagen, and the third using a Scaffold combination of chitosan and collagen. This study stated that in the Scaffold group the combination of collagen showed high OPG and osteoblast expression.	In this study concluded in the group scaffold combination of collagen and chitosan shows high OPG and osteoblast expression and is a high bone healing trigger material.
16.	Soetamans L, et all. 2020	Characteristics of chitin extracted from black soldier fly in different life stages	The BSF pupa shell has the most chitin content, which is 8% -24%. The purified chitin was subjected to physicochemical evaluation based on FTIR, XRD, and TGA as well as the deacetylation stage. The data shows that BSF chitin is α-chitin with an FTIR profile that closely matches shrimp chitin and shows some	Based on the FTIR, XRD and TGA spectra tests, the chitin extracted from each BSF life was designated as α-chitin. The FTIR spectra of commercial BSF and shrimp chitin samples matched well. The slight differences in physicochemical characters between chitin samples do not greatly affect further chitosan processing leading to the advantage that all parts of the BSF life that contain chitin can be

ISSN: 0005-2523
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			differences compared to squid pen chitin (β-chitin).	collected and processed into chitosan.
17.	[29]	Extraction of chitin and chitosan black soldier fly (Hermetia illucens) prepupa phase on characterization and yield.	BSF in each phase will have a different nutritional content and exoskeleton, in the prepupa phase it has a high protein content and a rather hard exoskeleton indicating the presence of chitin and chitosan components. In order to obtain chitin, the chitosan extraction process is carried out. The process also has differences in producing chitin, including the stages of demineralization and deproteination. while the manufacture of chitosan includes the stages of demineralization, deproteination, depigmentation and deacetylation.	The BSF prepupa phase chitin extraction yielded a yield of 18.05% and the characterization was blackish brown, while the BSF prepupa phase chitosan extraction produced a yield of 10.85% and characterization is white (pure).
18.	Bhavsar PS, et all. 2021	Sustainable Superheated Water Hydrolysis of Black Soldier Fly Exuviae for Chitin Extraction and Use of the Obtained Chitosan in the Textile Field	The amount of chitin obtained by BSF was 20% on the dry weight of BSF, this result is relatively higher with respect to the amount found in this study by Smets et al. In Larvae 3.85%, Prepupa 4.72%, and Pupa BSF 6.31%. Likewise, [13] determine the chitin content in larvae 3.6%, prepupae 3.1%, pupae 14.1%, and adult BSF 2.9%.	The purity of the chitin obtained, namely type α, was assessed by FTIR spectroscopy and thermal analysis, as well as the purity of the chitosan produced by deacetylation of chitin.

4. Discussion

The development of the times related to foods that contain protein sources has used insects as an alternative protein source. The use of black soldier fly pupae is mostly used as food for fish or livestock as a nutrient because the protein content is quite high, namely 44.26%, protein has a role in the absorption of Ca deposited on bones. Species that have been studied in terms of protein content may not necessarily be acceptable as a delicacy. BSF (Hermetia illucens) has the following taxonomic classification: Kingdom: Animalia Filum; Class Arthropoda: Insects; Order: Diptera; Family: Stratiomyidae; Subfamily: Hermetiinae; Genus: Hermetia; Species: Hermetia illucens. Order Diptera is the fourth most consumed order by humans. The commercial use of food for human BSF larvae (maggot) can be processed into foods that contain high protein, one of which is black soldier fly flour. BSF flour is quite suitable as a feed ingredient because it contains amino acids, fats and calcium. The amino acid profile contained in maggot flour in BSF is similar to soybean flour, especially the content of methionine or methionine + cystine which are essential amino acids for growth. According to several studies, BSF larvae are able to reduce the content of Escherichia coli and Salmonella enterica Serovar Enteritidis in chicken manure. So that the consumption of BSF larvae can be an alternative to adding protein to the human body with a good and proper processing system. Some examples that have been started in Sumedang City, West Java, Indonesia are processing it into BSF larvae chips and also BSF shredded larvae. This product still needs development, but so far it has been tried on several panelists including children, that the taste of processed BSF larvae can be accepted by the human sense of taste. This product still needs further research regarding other aspects such as business strategy, marketing, packaging, more attractive product forms [14], [15].

The tendency of people to use traditional medicine is getting higher, including several types of plants and animals that are used as traditional medicines for alternative wound healing. Utilization Black Soldier Fly (Hermetia illucens) can be a source of alternative medicine at this time. According to several studies, BSF pupae contain chitin which can be converted into chitosan. Chitosan is a natural polysaccharide synthesized from chitin extracted from the shells of crustaceans and insects. In general, insects that are used as a source of chitin are shrimp and crabs. However, there are several studies studying the extraction of chitin from other sources such as squid, cuttlefish, lobster, crayfish, beetles and silkworms. The BSF fly (Hermetia illucens), which is also known as the black soldier fly, is a fly originating from America and its larvae have been widely used as waste treatment, especially organic waste. The BSF life phase starts from larva, prepupa, pupa to adult flies. In the prepupa phase until they become pupae, BSF flies can be used as a potential source of chitin because the exoskeleton of the BSF flies contains as much as 35% chitin. Chitosan is multipurpose, widely used in the field of industry and health because of excellent biological and chemical properties, in the field of dentistry is used as an ingredient of root channels, antibacterial, wound healing or bone regeneration, and is used to improve the properties of other dental medicine [3- 5], [31], [32].

5. Conclusion

The purity of BSF chitosan is proven through the FTIR, XRD and TGA functional groups which are recognized by FDA (Florida Dental Association). Chitosan is a bioactive material in bone tissue engineering which can be processed in the form of a gel or scaffold. Chitosan improves osteogenic performance so that it can be used as a material for the treatment of bone defects by increasing the expression of Osteporotegerin (OPG), osteocalcin, BMP-2, osteoblasts and decreasing proinflammatory cytokines IL-6, TNF-α, RANKL which have all achieved good therapeutic effects.

6. Bibliography

[1] Gupta A, Rattan V, Rai S. Efficacy of Chitosan in Promoting Wound Healing in Extraction Socket: A Prospective Study. Journal of Oral Biology and Craniofacial Research. 2019; 9(2019): 91-95.

[2] Kurniawati E., Putranta. Potential of Chitosan Biopolymers in Wound Treatment. medulla. 2019; (9) 3: 459-456.

[3] Marsela L, Mangungsong S. The effect of maggot methanol extract (H. Illucens) on open wound healing in mice (Rattus novergicus). Pharmacy Health Journal (JKPharm). 2021; 3(2): 1-6.

[4] Sastro Y. Composting Technology of Municipal Organic Waste Using Black Soldier Fly. Jakarta Agricultural Technology Study Center (BPTP).

[5] Sulistiawaty L, Foliantini, Nurdiani, Puspita F. Isolation and Characterization of Chitin and Chitosan from Pupa Black Soldier Fly (BSF). Akab news. 2022; (46) 1: 56-62.

[6] Rahmitasari F. Scaffold 3D chitosan and collagen as grafts on cases of bone damage (literature study). Journal of Dentistry Materials. 2016; 5(2): 1-7.

[7] Pratiwi, SN. Utami N, Damayanti PN. Extraction of Chitosan and Characterization of Nanoparticle Chitosan from Pupae Sheels of Black Soldier Fly (Hermetia illucens). Medical Science: Pharmaceutical Scientific Journal. 2022; 7(4): 936-946.

[8] Kurniawati, A., Wahyukundari, MA, & Astuti, SD (2020). The potential of purple leaf extract in reducing the number of osteoclast cells induced by porphyromonas gingival. Cakradonya Dental Journal, 12(2), 75-82.

[9] Soeroso Y, Bachtiar EW, Bachtiar Boy M, Sulijaya B, Prayitno SW. The Prospect of chitosan on the osteogenesis of periodontal ligament stem cells. J Int Dent Med Res. 2016;9(2):93–7.

[10] Chaesaria G, Hasan M, Narwanto MI. The Effect of White Shrimp Shell Chitosan (Penaeus meguiensis) on the number of osteoblast cells of the female mice after ovaryectomy. e-Journal of Health Library. 2015; 3(3): 375-379.

[11] Achmad, H., Gani, A., Djais, A., Hatta, LI, Rieuwpassa, IE, & Monry, AYAA "Effectiveness of edible film chitosan from waste white shrimp (Litopenaeus vannamei) in reducing the colonization of porphyromonas gingivalis bacteria: In vitro research , Annals of the Romanian Society for Cell Biology (2021): 1673-1681

[12] Ramadhani T, Parwati Sari R. Effectivity of Combination of Sardine Fish Oil (Sardinella longiceps) and Hydroxyapatite Application on The Expression of FGF-2in Bone Healing Process. 2016;10(1).

[13] Wang YS, Shelomi M. Review of Black Soldier Fly (hermetia Illucens) as Animal Feed and Human Food. Food. 2017; 6(10): 91.

[14] Yuwono AS, Mentari PD. The use of larvae (maggot) black soldier fly (BSF) in managing organic waste. Seameo Biotrop. 2018: 44.

[15] Gani A et al. Effectiveness of Combination of Chitosan Gel and Hydroxyapatite from Crabs Shells (Portunus pelagicus) Waste as Bonegraft on Periodontal Tissue Regeneration through IL-1 and BMP-2 Analysis. Hindawi International Journal of Biomaterials. 2022(4);1-8

[16] Bashir SM, Rather GA, Haq Z, Sheikh AA, Shah MZUH, Singh H, Khan AA, Imtiyaz S, Ahmad SB, Nabi S, Rakhshan R, Hassan S, Fonte P.Chitosan Nanoparticles: A Versatile Platform for Biomedical Applications. Materials. 2022; 15(19): 6521.

[17] DA spider. The Validity of Nano-Chitosan/Nano-Hydroxyapatite As A Promoter Of Bone Healing In Ovariectomized Rats. Egyptian Dental Journal. 2017; 63(3): 2389-2402.

[18] Rostini R, Kuntjoro M, Sitalaksmi RM, Salim S. Spirulina Chitosan Gel Induction on Healing

Process of Cavia Cobaya Post Extraction Socket. DentalJournal. 2014; 47(1): 19-24.

[19] Pratiwi, SN. Utami N, Damayanti PN. Extraction of Chitosan and Characterization of Nanoparticle Chitosan from Pupae Sheels of Black Soldier Fly (Hermetia illucens). Medical Science: Pharmaceutical Scientific Journal. 2022; 7(4): 936-946.

[20] Kamadjaja MJK. Bone remodeling using a three-dimensional chitosan - hydroxyapatite scaffold seeded with hypoxic conditioned human amnion mesenchymal stem cells. Dental Journal 2021; 54(2): 68–73.

[21] Yousefiasl S et al. Bioactive 3D-printed chitosan-based scaffolds for personalized craniofacial bone tissue engineering. KeAi Chinese Roots Global Impact. 2023; 4(2023):1-11

[22] Gani A et al. The Effect of White Shrimp Head Chitosan Gel (Litopenaeus vannamei) on Inhibitory Strength of Periodontopathogenic Bacteria and Accelerating Wound Healing (In Vitro, Histological, and Clinical Tests). Sys Rev Pharm 2020; 11(4): 258 267.

[23] Jayash et al. Analysis on Efficacy of Chitosan-based Gel on Bone Quality and Quantity. 2021; 8(640950):1-10

[24] Santhosh S et al. Improved treatment efficacy of risedronate functionalized chitosan nanoparticles in osteoporosis: formulation development, in vivo, and molecular modeling studies. 2019; 36(4):338-355

[25] Chatzipetros E et al. The effect of nano-hydroxyapatite/chitosan scaffolds on rat calvarial defects for bone regeneration. International Journal of Implant Dentistry. 2021; 7(40): 1-11

[26] Nuntanaranont T, Boundmanatham B, Kamolmatyak S. Effect of chitosan scaffold on bone healing in rabbit calvarial defects. Songklanakarin J. Sci. Technol. 2018 40(4), 792-797.

[27] Winias S, Ernawati DS, Ariani MD, Rahayu rp. Scaffold combination of chitosan and collagen synthesized from chicken feet induces osteoblast and osteoprotegerin expression in the bone healing process of mice. Dental Journal of Dentistry. 2017; 50(2):86-90

[28] Soetemans L, Uyttebroek M, Bastiaens L. Characteristics of chitin extracted from black soldier fly in different life stages. International Journal of Biological Macromolecules. 2020; 3206-3214.

[29] Mirwandhono E, Nasution MIA, Yunilas. Extraction of chitin and chitosan black soldier fly (Hermetia illucens) prepupa phase on characterization and yield. IOP Conf. Series: Earth and Environmental Science. 2019:012019.

[30] Lestari W, Yusry WNAW, Haris MS, Jaswir I, Idrus E. A glimpse of the function of chitosan as a dental hemostatic agent. Japanese Dental Science Review. 2020 Nov 1;56(1):147–54.

[31] Tri Hartomo B, Griselda Firdaus F. Utilization of Chitosan Biomaterials in Oral Surgery Keywords Abstract. B-Dent: Baiturrahmah University Journal of Dentistry [Internet]. 2019;6(1):62–70. Available from: https://jurnal.unbrah.ac.id/index.php/bdent/index

ISSN: 0005-2523
Volume 63, Issue 06, June, 2023

[32] Aguilar, A et al. (2019). Application of chitosan in bone and dental engineering. Molecules, 24(16), 3009.

[33] Djais, AI, Mappangara, S., Gani, A., & Achmad, H. (2023). The Use Of Chitosan Milkfish (Chanos Chanos) Scales Waste As An Alternative Bone Regeneration Material In Socket Preservation. Journal of Pharmaceutical Negative Results, 3737-3748.

[34] Mohan, K., Ganesan, AR, Muralisankar, T., Jayakumar, R., Sathishkumar, P., Uthayakumar, V and Revathi, N. (2020). Recent insights into the extraction, characterization, and bioactivities of chitin and chitosan from insects. Trends in food science & technology, 105, 17-42.

[35] Maryani, I., Rochmah, YS, & Parmana, AD (2018). Analysis of Platelet Rich Plasma and Chitosan Combination Gel on Increasing Number of Osteoblasts as Bone Regeneration in Wistar Rats Post Tooth Extraction Wounds. Odonto: Dental Journal, 5(2), 89-96.

[36] Tian, Y., Wu, D., Wu, D., Cui, Y., Ren, G., Wang, Y and Peng, C. (2022). Chitosan-based biomaterial scaffolds for the repair of infected bone defects. Frontiers in Bioengineering and Biotechnology, 755.

[37] Hahn, T., Roth, A., Febel, E., Fijalkowska, M., Schmitt, E., Arsiwalla, T., & Zibek, S. (2018). New methods for high-accuracy insect chitin measurement. J. Sci. FoodAgri. 98(13), 5069–5073.

[38] Wasko, A., Bulak, P., Polak-Berecka, M., Nowak, K, Polakowski, C., & Bieganowski. (2017). The first report of the physicochemical structure of chitin isolated from Hermetia illucens. int. J. Biological Macromolecules, 92, 316–320.

[39] Kadouche, S., Farhat, M., Lounici, H., Fiallo, M., Sharrock, P., Mecherri, M., et al. (2016). Low Cost Chitosan Biopolymer for Environmental Use Made from Abundant Shrimp Wastes. Waste Biomass Valor 8 (2), 401–406. doi:10.1007/ s12649-016-9593-2.

[40] Kanto, DAR, Permana, AD, & Hertadi, R. (2019). Extraction and characterization of chitin and chitosan from the black soldier fly (Hermetia illucens). Marine Pharmacopoeia Scientific Journal, 10(1), 23-32.