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Investigation of Bioactive Compounds from Sea Cucumber Extract and Stingless Bee Honey Using Critical Literature Review and Bioinformatics Approach for Potential Synergy Combination

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Abstract: Stingless bee and sea cucumber can both be found locally in Malaysia, with local species. One major product that has been commercialized on a large scale in Malaysia from stingless bees is the stingless bee honey, commonly known as kelulut. There is increasing research that shows the benefits of kelulut honey for human health, including its potential use in managing blood sugar levels for diabetes and obesity prevention. Meanwhile, sea cucumber has also been traditionally consumed as a food delicacy in Malaysia, and Malaysian sea cucumbers are being exported in dried form worldwide for food consumption. Besides its nutritious composition, several sea cucumber species such *Stichopus horrens* or commonly called 'gamat emas' by Malaysians are traditionally used for wound healing purposes in the forms of oil for external use or ingested in liquid form for internal use. Published studies demonstrated that sea cucumbers have bioactive compounds that are beneficial for human health. In this study, the feasibility of a synergy between sea cucumber extract and stingless bee honey for positive medical and health impact will be investigated using bioinformatics approaches and critical literature reviews. Critical literature review on bioactive compounds from sea cucumbers and stingless bee honey as well as potential synergy combinations were performed using Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Twenty-seven sea cucumber glycoproteins were selected for *in silico* bioinformatics analysis to undergo physicochemical analysis, secondary and tertiary protein model prediction, and protein-ligand docking. The study described the bioactive compounds from sea cucumbers and stingless bee honey, most of which have experimental

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evidence to support their reported beneficial activity for human benefit. Bioinformatics analyses performed in this study successfully produce 3D protein models for 25 glycoproteins from *Apostichopus japonicus*. Preliminary protein-ligand docking between the glycoprotein models with their predicted ligands has been performed. These bioinformatics data will be useful for the future *in silico* molecular dynamics simulation to look further into the potential uses of these glycoprotein targets. The potential of producing a novel functional food combining sea cucumber extract with stingless bee honey is also discussed along with future synergy experiments that can be performed to test this hypothesis. Based on the findings from critical literature review and bioinformatics analyses, both sea cucumber and stingless bee honey are beneficial to human lives and should be sustainably utilized by humans. It is hoped that this study could be a starting point to investigate the potential of combining kelulut honey with sea cucumber extract as a novel functional supplement.

Keywords: Kelulut, Stingless Bee Honey, Sea Cucumber, Gamat, Synergy

1. Introduction

Natural products are now being used as preventative treatments as well as being integrated or complement conventional medical treatments. This is because natural products generally contain low toxicity and cause less side effects, with some having sufficiently high potency to prevent or overcome infections. Therefore, natural products can be utilized in the discovery of new antimicrobial medicine and can also be used to overcome infections.

1.1 Honey as novel health supplements

Honey is a natural product which is produced by honeybees (*Apis sp.)* and stingless bee honey (Melipona and Trigona genera). Locally in Malaysia, the stingless bee is also known as *kelulut*, it produces different types of honey which contain higher polyphenols content than common honeybees [1]. Its properties of honey contain medicinal properties which have high potential to prevent chronic disease [2]. Honey has been consumed traditionally to treat mild diseases such as eye disease, throat infection, dizziness, and eczema. It is effective because the honey has medicinal properties such as antioxidant, antimicrobial, anti-inflammatory, anti-proliferative, anti-cancer, and anti-metastatic effects [3].

The composition of honey is formed from the collection of different substances such as nectar, essential oils, pollen, and various botanical origins. One of the highlighted ingredients in honey is hydrogen peroxide (H_2O_2) which contains medicinal properties which is antibacterial activity [4,5].

1.2 Sea Cucumber's medicinal properties

In Malaysia, the sea cucumber has been consumed for a long time as a traditional health supplement. There are many species of sea cucumber harvested for its nutritional value in Asian countries such as *Holothuria atra* and *Holothuria leucospilota*. Other than the nutritional content of sea cucumbers, sea cucumbers also contain a lot of bioactive compounds which have antibacterial properties such as saponins (triterpene glycosides) [6]. Compounds in sea cucumber i.e., chondroitin sulfate, sulfated polysaccharides, glycoprotein, essential fatty acids, glycosphingolipids, sterols peptides, and glycosaminoglycan (GAGs) can act as bio actives and provide medical and health benefits [7].

These marine invertebrates are unique as they contain high levels of protein, low fat and sugar content and they are cholesterol free. Sea cucumber is also rich in bioactive compounds which include anticancer, anti-angiogenic, anticoagulant, anti-inflammatory, antimicrobial, antioxidant, and could be helpful in healing wounds [8,9].

Using the commercial production of cough syrup which combines the sea cucumber extract and common bee honey as an example, the combination of sea cucumber extract and stingless bee honey could be a new novel health supplement. The stingless bee honey has been claimed to contain higher medicinal properties than common bee honey, in which it has the highest energy content, lowest moisture level, fat content and fructose level [10].

1.3 Problem statements

Nowadays, the resistance towards the antibiotic has been raising concerns to the society. The ability of antibiotics to treat disease has been affected due to the pathogenic resistance. To overcome these problems, a correct guideline to use antibiotics should be practiced. The natural sources such as honeybee and sea cucumber extract contained a lot of beneficial components which can be used as medicinal supplements.

As of today, a few combinations of natural products have been produced such as honey and black seed (common arabic name Habbatassauda'), honey and garlic combination and in Asia, honey and sea cucumber extract combination. In recent years, the synergism of stingless bee (*Trigona spp.*) or kelulut honey and ampicillin has been studied for its antibacterial activity against *Staphylococcus aureus*. Based on the study, the results on the synergism are successful. The combination of both materials causes a greater antibacterial activity against *S. aureus*. The combination of kelulut honey with ampicillin is used for wound infection to inhibit prolonged toxicity [11].

2. Methodology

2.1 In *silico* Bioinformatics Analysis

Sea cucumber *Apostichopus japonicus* is used because the fully sequenced genomes of *A. japonicus* has been published and is made available for public use in NCBI database.

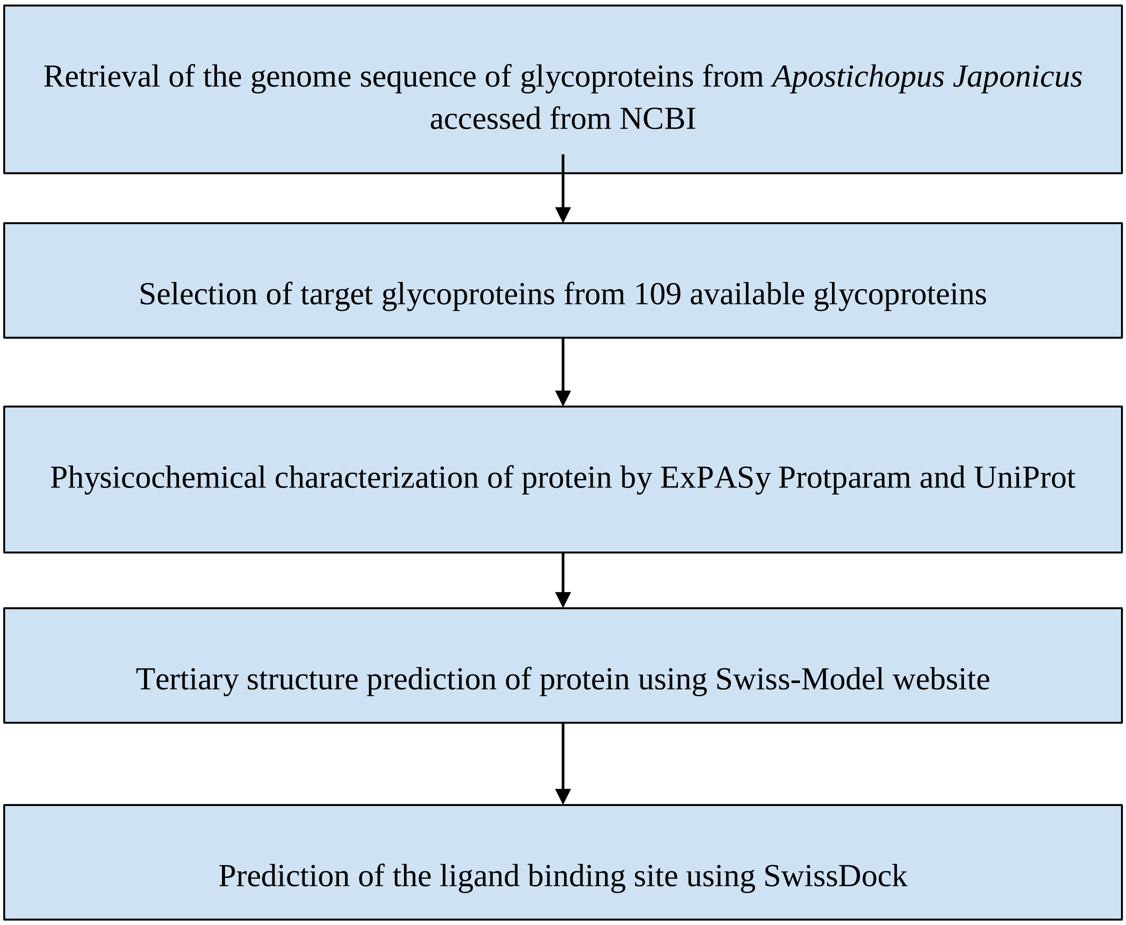


Figure 1: Flow charts of retrieving data for bioinformatics of sea cucumber

2.1.1 Genome sequence retrieval

The ID name of the *Apostichopus japonicus* protein is retrieved from National Centre for Biotechnology Information (NCBI) website (https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/12044/352579%7CApostichopus%20japonicus/Un/)

2.1.2 Physicochemical characterization

The physical and chemical parameters of protein (molecular weight, isoelectric point, extinction coefficient, instability index, and GRAVY) were analyzed using the ExPASy ProtParam tool (https://web.expasy.org/protparam/) [12].

2.1.3 Tertiary structure prediction

The protein tertiary structure was predicted using three different servers. These are the I-TASSER (https://zhanglab.dcmb.med.umich.edu/I-TASSER/) and ExPASy SWISS-MODEL (https://swissmodel.expasy.org/).

2.1.4 Ligand prediction

The active sites and ligands of tertiary structures prediction were predicted by I-TASSER (https://zhanglab.dcmb.med.umich.edu/I-TASSER/) and SwissDock (http://www.swissdock.ch/docking).

2.2 Critical literature review for Stingless bee honey

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines is used in this methodology [13]. The search servers used are using the online databases which are ScienceDirect, National Centre for Biotechnology Information (NCBI) and SpringerLink. The journal and article retrieved from the database will be filtered according to the objective.

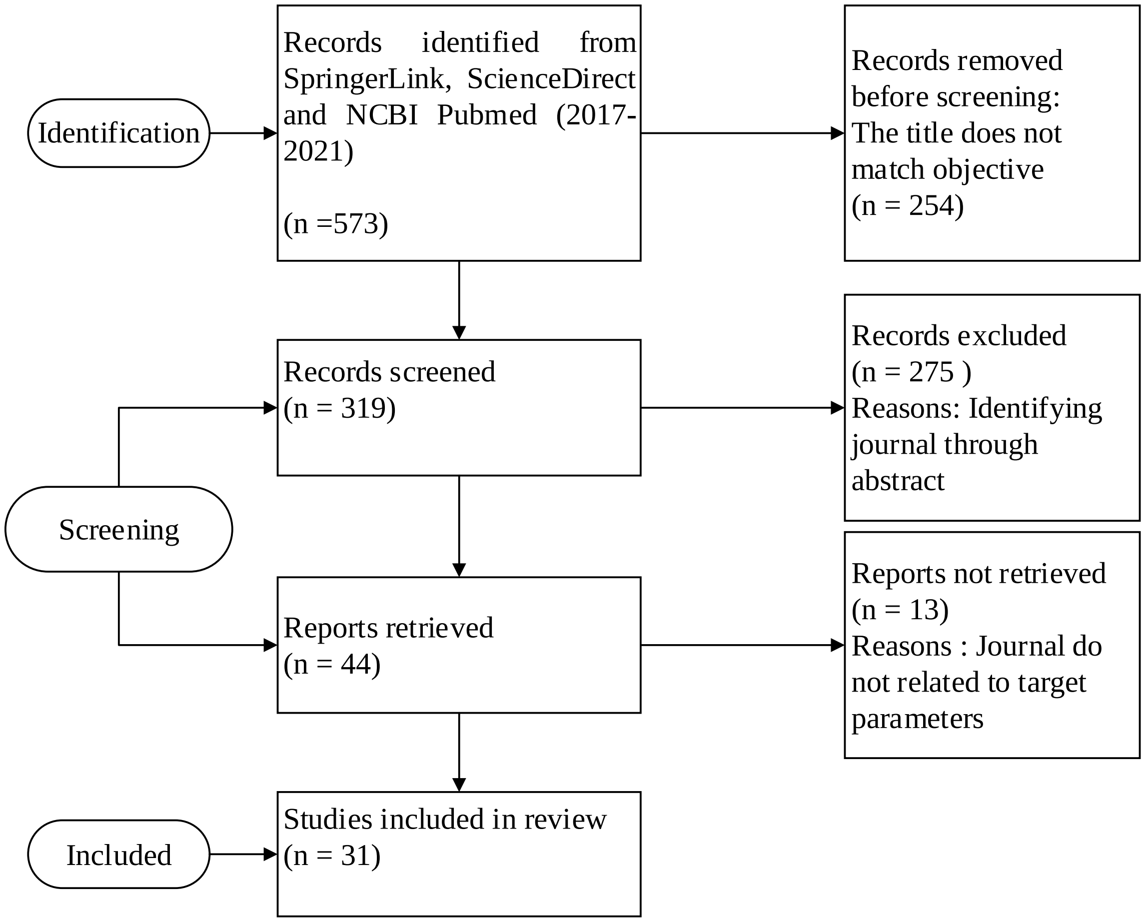


Figure 2: Flow charts of retrieving critical literature review for stingless bee honey

3. Results and Discussion

The genome sequence of *Apostichopus japonicus* is retrieved from the NCBI database. From the genomic data, 27 out of 109 glycoproteins were selected for *in silico* bioinformatics analysis. The protein name, molecular weight, theoretical point (pI), extinction coefficient, aliphatic index and GRAVY are the parameters of physicochemical characteristics that were recorded (Table 1).

As for the stingless bee honey, it is not feasible to perform bioinformatics experiments on stingless bee honey. Thus, critical literature reviews will be performed to study stingless bee honey properties and important functional groups to achieve the set objectives for this study.

3.1 Results of bioinformatics analysis on glycoprotein targets from sea cucumber *Apostichopus japonicus*

3.1.1 Physicochemical Characterization of Selected Glycoproteins from *Apostichopus japonicus*

Table 1 lists the selected glycoproteins targets from the genome sequence of *Apostichopus japonicus*. The physicochemical characteristics of the glycoprotein targets are recorded. The recorded data are important parameters to know for future purification and handling of the glycoproteins in the laboratory.

Table 1: Physicochemical characterization

No.	Protein ID	Molecular weight (Da)	pI point	Extinction coefficient	Aliphatic index	GRAVY
1	PIK58558.1	52295.87	5.83	30785	98.33	-0.115
2	PIK51533.1	75655.02	5.85	90480	97.72	-0.18
3	PIK51379.1	51169.67	4.69	48485	87.43	-0.299
4	PIK51475.1	35150.99	7.13	96175	67.54	-0.64
5	PIK43470.1	35722.03	8.81	46005	73.28	0.371
6	PIK47039.1	49115.82	4.26	103220	46.98	-0.615
7	PIK59004.1	64745.09	8.99	69010	80.02	-0.481
8	PIK43097.1	84579.35	5.75	102400	77.51	-0.383
9	PIK47848.1	17477.68	9.19	31315	103.16	-0.494
10	PIK62660.1	37077.98	6.34	26150	84.82	-0.383
11	PIK41500.1	39141.75	6.44	19545	60.86	-0.577
12	PIK41283.1	17924.33	9.5	25900	67.08	-0.722
13	PIK58116.1	15934.31	5.88	21025	76.67	-0.115
14	PIK41282.1	16370.92	9.3	10095	89.31	-0.062
15	PIK39729.1	44178.52	7.3	66750	55.41	-0.545
16	PIK58114.1	14943.27	6.79	8075	76.17	-0.095
17	PIK58117.1	14746.07	7.53	19200	89.39	0.058
18	PIK39609.1	39969.64	9.55	70150	106.02	0.303
19	PIK38549.1	20708.27	4.93	21345	130.7	0.542
20	PIK54185.1	158249.38	5.95	112495	93.96	-0.1
21	PIK53667.1	48132.78	5.46	55600	85.47	-0.311
22	PIK53438.1	33018.53	4.55	51295	73.25	-0.459
23	PIK43734.1	20847.63	4.06	6210	75.03	-0.35
24	PIK39607.1	14392.84	5.15	32220	118.24	0.584
25	PIK56460.1	28325.62	6.37	12170	110.56	0.065
26	PIK43470.1	35722.03	8.81	46005	73.28	-0.371
27	PIK43097.1	84579.35	5.75	102400	77.51	-0.383

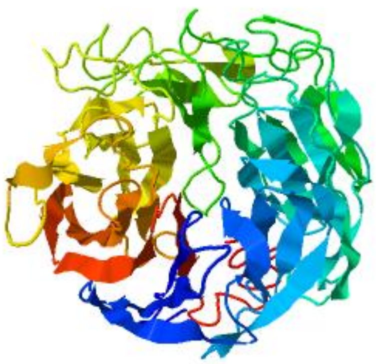
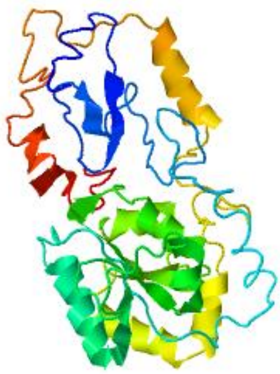
3.1.2 Secondary and Tertiary Structure Prediction of Selected Glycoproteins

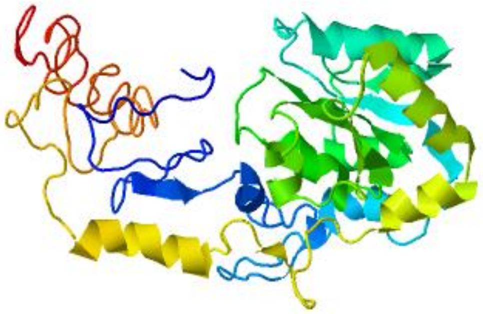
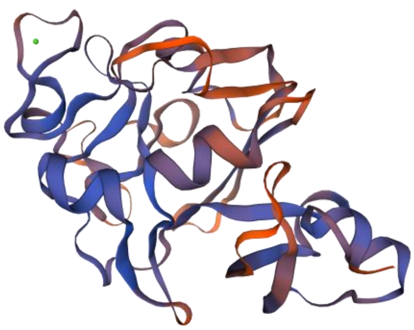
Glycoproteins are molecules that are composed of protein and carbohydrate chains. Glycoproteins are a large group of biomolecules, with a diversity of functions. The formation of attachment between glycans and protein is through the glycosylation process, e.g. N-glycosylation (attachment of glycans to nitrogen on the amine side chain of amino acid) and O-glycosylation (attachment of glycans to oxygen on an amino acid). One of the most studied glycoproteins from marine organisms that has biological relevance is lectins. Lectins isolated from red algae *Solieria filiformis* and red seaweed *Bryothamnion triquetrum* showed anti-inflammatory effects, while lectins isolated from sea mollusk *Crenomytilus grayanus* and shrimp *L. vannamei* displayed anti-cancer activity. Antiviral action was also observed in lectins isolated from the green alga *Halimeda renschii* [14].

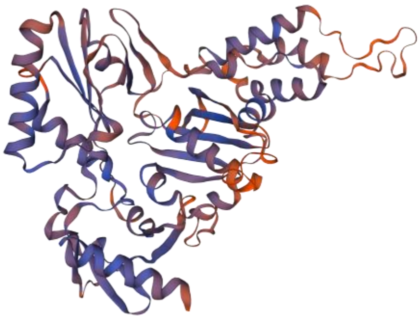
Glycoproteins and chondroitin are the key components found in dried sea cucumber preparations. A 2016 study by Korean scientists showed that glycoproteins from sea cucumber cooked liquid has no cytotoxicity effects on normal mouse melanoma cell line suspensions, with a significant tyrosinase inhibition activity (suppressing melanin synthesis useful for skin whitening products) and significant elastase inhibitory activity (inhibiting elastase activity thereby useful to develop cosmetics with anti-corrugation ingredients for reducing wrinkles formation) [15]. These findings indicate the potential of sea cucumber being used as a cosmetics material.

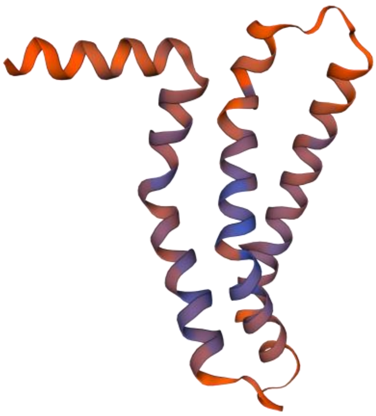
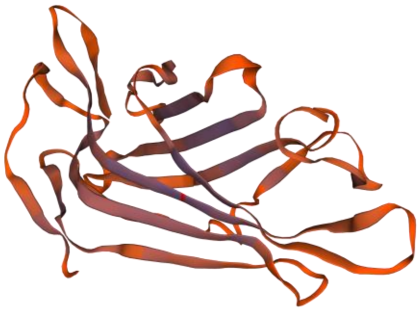
There are 109 glycoproteins found in the genome of *Apostichopus japonicus*. 62 of them are putative glycoprotein 3-alpha-L-fucosyltransferases, 10 are putative thy-1 membrane glycoprotein isoform X1 and 6 are putative microfibril-associated glycoprotein 4.

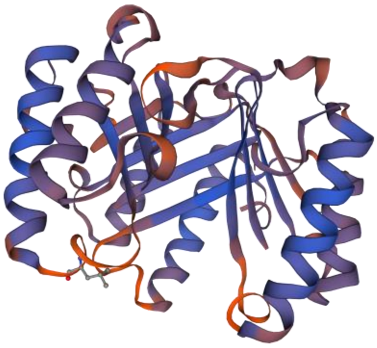
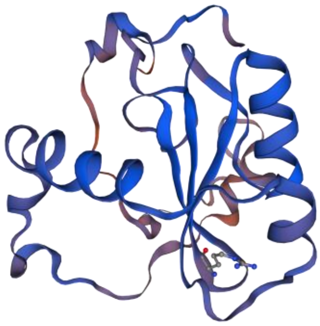
From the 27 selected glycoproteins targeted for bioinformatics analysis, 25 glycoproteins could be modelled (Figure 3).

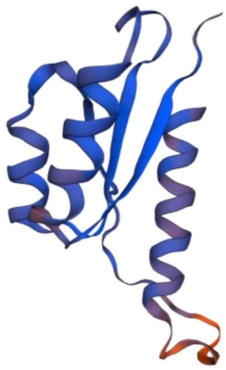
1)	Putative mesenchyme-specific cell surface glycoprotein (PIK51379.1)	2)	Putative glycoprotein 3-alpha-L-fucosyltransferase A-like (PIK51475.1)
			

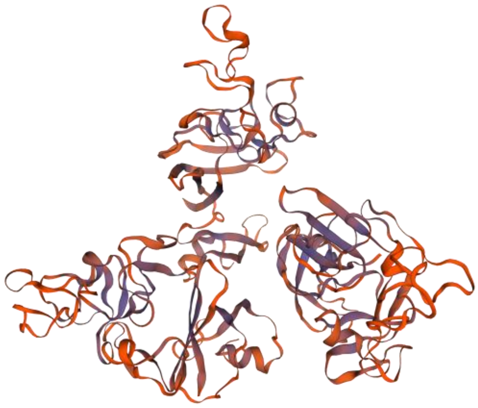
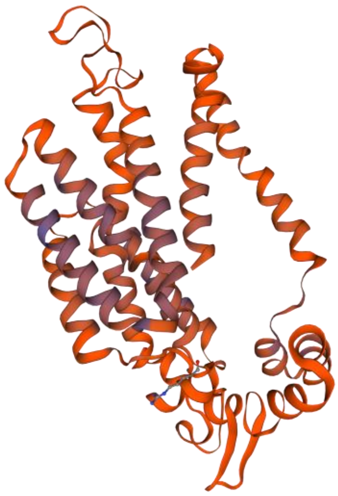
3	Glycoprotein 3 alpha L fucosyltransferase (PIK43470.1)	4	Putative microfibril-associated glycoprotein 4 (PIK47039.1)
			
5	Putative thy-1 membrane glycoprotein isoform X1 (PIK59004.1)	6	Putative glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase 1-like (PIK43097.1)

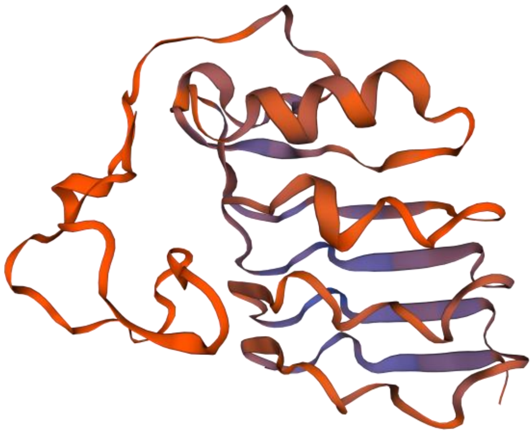
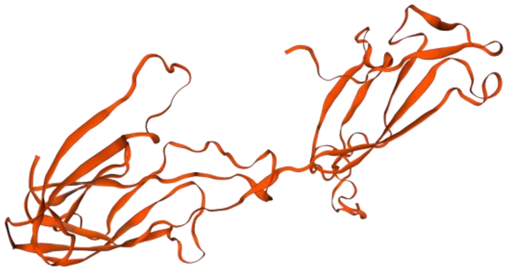
			

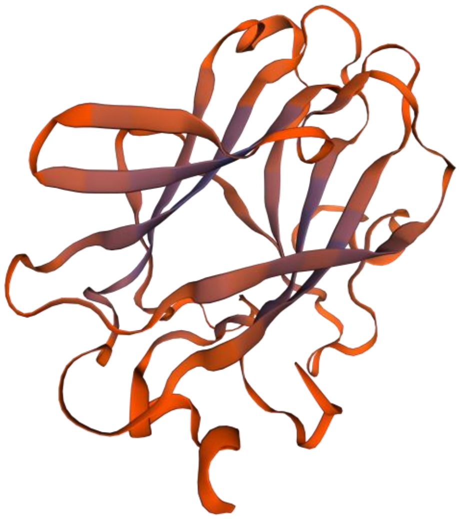
7	Glycoprotein hormone receptor (PIK47848.1) 	8	Putative lysosome-associated membrane glycoprotein 5 (PIK62660.1) 
9	Putative cell surface glycoprotein 1-like (PIK41500.1)	10	Alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase (PIK41283.1)

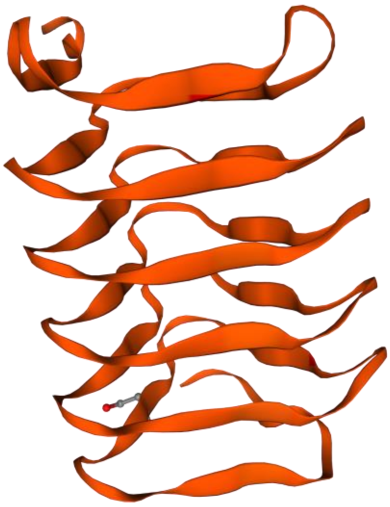
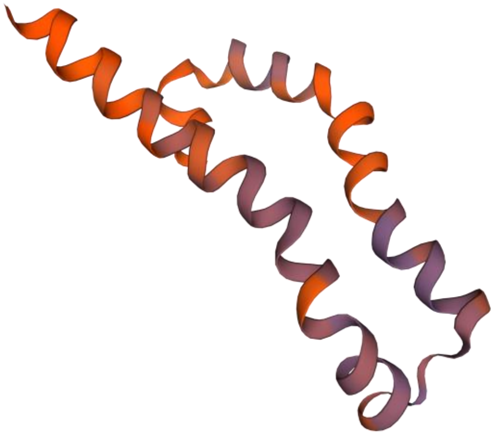
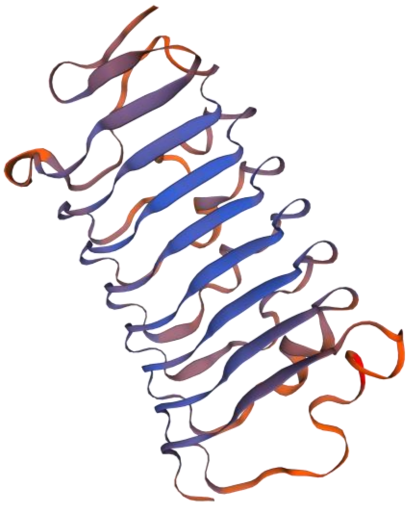
			

11	Putative glycoprotein hormone beta-5 (PIK58116.1) 	12	Putative alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase, partial (PIK41282.1) 
13	Putative microfibril-associated glycoprotein 4-like (PIK39729.1)	14	Putative glycoprotein hormone-beta5 (PIK58114.1)

			
15	Putative glycoprotein hormone-alpha2 (PIK58117.1) 	16	Putative synaptic vesicle glycoprotein 2C isoform X2 (PIK39609.1) 
17	Putative leucine-rich alpha-2-glycoprotein (PIK38549.1)	18	Putative nuclear pore membrane glycoprotein (PIK54185.1)

19	Putative alpha-1,3-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase C-like (PIK53667.1) 	20	Putative apical endosomal glycoprotein (PIK53438.1) 
21	Glycoprotein X (PIK43734.1)	22	Synaptic vesicle glycoprotein 2C, partial (PIK39607.1)

			
23	Putative platelet glycoprotein Ib alpha chain (PIK56460.1) 	24	Glycoprotein 3 alpha L fucosyltransferase (PIK43470.1) 
25	Putative glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase 1-like (PIK43097.1)		

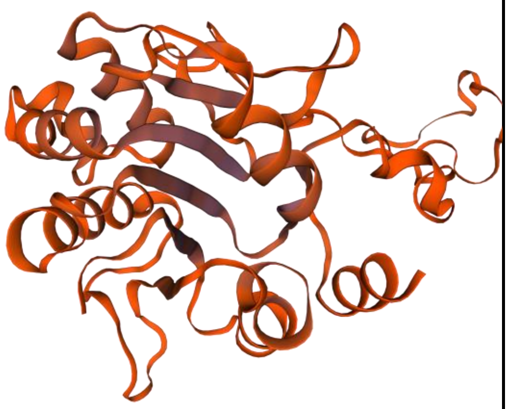


Figure 3: Tertiary structure ribbon model of 25 glycoproteins from *Apostichopus japonicus*

3.2 Other bioactive compounds from sea cucumber

3.2.1 Polysaccharides

3.2.1.1 Sulfated polysaccharides

Sulfated polysaccharides are negatively charged polysaccharides present in many different groups of organisms, from macroalgae to mammals, with the exception of land plants. Seaweeds, including red (Rhodophyta), green (Chlorophyta), and brown (Phaeophyceae) marine macroalgae, biosynthesize sulfated polysaccharides as a key component of their cell walls. Sulfated polysaccharides have been extensively studied in seaweeds, with well-known sulfated polysaccharides in seaweeds include galactans (agarans and carrageenans) from red algae, ulvans from green algae, and fucans and fucoidans from brown algae [16].

A search in NCBI Pubmed for "Sulfated AND polysaccharides AND cucumber" (the term 'sea' in 'sea cucumber' is removed to reduce the addition of other sea organisms in the search) revealed 99 hits. Using manual removal, 75 articles were excluded (which reported on fucosylated chondroitin sulfate), leaving behind 24 journal articles on sulfated polysaccharides. From the 24 articles, five demonstrated anti-coagulant activities of purified sulfated polysaccharides isolated from various sea cucumber species [17, 18, 19, 20, 21], followed by a report of immune-enhancing properties of a purified sulfated polysaccharide isolated from sea cucumber viscera [22], modulation of the gut microbiota and its metabolites in normal mice [23], prevention of diet-induced obesity with modification of gut microbiota in high-fat diet-fed mice [24], anti-cancer activity in vitro [25], inhibition of the Aβ40 aggregation (as a mean to prevent and treat Alzheimer's disease) [20], natural killer cells activation and cytotoxicity [26], and amelioration type 2 diabetes in rats (potential to develop hypoglycemic functional food for diabetes) [27], among others.

A review paper recently summarized the pharmacologic potential of sea cucumbers, and reported on bioactive compounds with experimentally confirmed pharmacological activity from sea cucumbers, including sea cucumbers polysaccharides (sulfated fucan, fucosylated chondroitin sulfate) [28].

A recent study demonstrated the significant inhibitory activities of sea cucumber (*Stichopus (Apostichopus) japonicus*) sulfated polysaccharide against SARS-CoV-2 virus, along with fucoidan from brown algae, iota-carrageenan from red algae. Sea cucumber sulfated polysaccharide demonstrated the highest inhibitory activity with IC50 of 9.10 μg mL-1. Furthermore, a test using pseudotype virus with S glycoprotein showed that sea cucumber sulfated polysaccharide could also bind to the S glycoprotein to prevent SARS-CoV-2 host cell entry (potential to develop as antiviral drugs against SARS-CoV-2 infection) [29].

3.2.1.2 Fucosylated chondroitin sulfate

Fucosylated chondroitin sulfate (fCS) is a distinct marine glycosaminoglycan (GAG) found exclusively in sea cucumbers (Echinodermata, Holothuroidea). Glycosaminoglycans (GAGs) are linear and heterogeneous sulfated glycans [14]. The body walls of sea cucumbers contain two main types of sulfated polysaccharides, sulfated fucans (SF) often named "fucoidans", and fucosylated chondroitin sulfates (fCS). The latter polysaccharides isolated from different species of sea cucumbers are known to be composed of d-glucuronic acid, *N*-acetyl-d-galactosamine, l-fucose and sulfate residues [30].

A published study performed anti-inflammatory assays on fucosylated chondroitin sulfate from sea cucumber *Acaudina molpadioidea* in China, and the results showed a significant reduction of the carrageenan induced edema in a dose dependent manner. This indicates the potential of utilizing sea cucumber as a potential antiallergic agent [31].

In another study, Russian researchers compared anti-inflammatory activity of fucosylated chondroitin sulfate (fCS) from sea cucumber *Cucumaria djakonovi* to chondroitin sulfate from cartilage of the fish *Salmo salar*. Based on a model of acute peritoneal inflammation in rats, the results showed 45.00 % inhibition of inflammation by fCS from sea cucumber compared to 31.00 % inhibition by chondroitin sulfate from the fish. The presence of sulfated fucosyl branches is essential for the anti-inflammatory effect of chondroitin sulfates of marine origin [30]. The same group of researchers also performed anticoagulant assay using clotting time assay on fucosylated chondroitin sulfate from two sea cucumber species: *Paracaudina chilensis* and *Holothuria hilla*. Both polysaccharides demonstrated significant anticoagulant activity [32].

A review paper on chondroitin [33] reported on anti-angiogenesis and anticoagulation properties of isolated fucosylated chondroitin sulfate from the body wall of the sea cucumber *Holothuria mexicana* [34], anticoagulation and antithrombosis properties from isolated fucosylated chondroitin sulfate of sea cucumber *Cucumaria frondosa* [35], and a novel function of the promotion of neurite outgrowth (development of neurons and nerve regeneration) from fucosylated chondroitin sulfate isolated from the cell wall of *Apostichopus japonicus* [36].

From the genome sequence of *Apostichopus japonicus,* there are 8 proteins that are directly related to chondroitin sulfate (Table 2). For this study, these 8 proteins were not analysed in more detail using bioinformatics because the fucosylated chondroitin sulfate has been identified to be an important bioactive compound. In future study, these 8 proteins can be studied further to understand the synthesis and metabolic pathway to produce chondroitin sulfate.

Table 2: Eight genes with direct relationship to chondroitin sulfate

No	Protein Name	Protein ID
1	Putative chondroitin sulfate N-acetylgalactosaminyltransferase 1 isoform X2	PIK32884.1
2	Putative chondroitin sulfate N-acetylgalactosaminyltransferase 1	PIK32938.1
3	Putative chondroitin sulfate synthase 1	PIK39716.1
4	Putative chondroitin sulfate synthase 1	PIK41082.1
5	Putative chondroitin sulfate proteoglycan 4	PIK41082.1

6	Putative chondroitin sulfate synthase 1	PIK42138.1
7	Putative chondroitin sulfate synthase 1	PIK42139.1
8	Putative chondroitin sulfate synthase 2	PIK55628.1

3.2.1.3 Triterpene glycosides

Triterpene glycosides are present in many plants, fungi and marine organisms. Triterpene glycoside indicates the presence of three terpene units, attached to a glycoside, a sugar moiety. More than 300 triterpene glycosides have been isolated and characterized from various species of sea cucumbers, and thus, they are characteristic secondary metabolites of echinoderms, as well as octocorals, and sponges [37]. Triterpene glycosides contain a carbohydrate chain up to six monosaccharide units mainly consisting of d-xylose, 3-*O*-methy-d-xylose, d-glucose, 3-*O*-methyl-d-glucose, and d-quinovose. Cytotoxicity is the common biological property of triterpene glycosides isolated from sea cucumbers. They act as biological toxins to protect them from eukaryotic predators, which explain why they do not possess antibacterial activity. Besides cytotoxicity, triterpene glycosides also exhibit antifungal, antiviral, and hemolytic activities. A review on sea cucumber glycosides has reported on the various types of sea cucumber glycosides that have been shown to be able to exhibit biological activities in both in vitro and in vivo models [38]. Due to these unique characteristics however, triterpene glycosides from sea cucumber can be utilized for development of drugs for human benefits.

3.3 Stingless bee honey

3.3.1 Physicochemical analysis

The results of moisture content, pH, free acidity, total soluble solids (TSS), colour, and 5-hydroxymethylfurfural are shown in Table 3.

Table 3: Parameters of stingless bee honey of different botanical origins and bee honey [39]

Parameters	Botanical Origins			*Apis melifera*
	Acacia	Starfruit	Gelam	
Moisture content	21.52 ± 0.66	24.24 ± 0.19	25.49 ± 0.45	14.67 ± 0.11
pH	3.27 ± 0.03	3.00 ± 0.03	3.18 ± 0.03	2.56 ± 0.02
Free acidity	107.50 ± 6.45	246.65± 9.46	176.25 ± 9.46	39.22 ± 1.50
TSS	74.65 ± 0.39	73.88 ± 0.34	74.85 ± 0.73	76.40 ± 0.54
5-HMF	ND	0.07 ± 0.06	0.05 ± 0.02	ND

Based on the result, the stingless bee honey has the best properties compared to the common honey *Apis melifera*. According to Keng et al., it is suggested that the moisture content of honey does not exceed 20 g/100 g. This is mostly because honey is susceptible to fermentation and has poor antimicrobial stability when the moisture level exceeds 20 g/100 g [40]. The natural properties of honey are acidic, ranging from 3.2 to 4.5. However, the stingless bee honey is known for its low pH values which ranges from 3.15 to 6.64. In addition, its low pH value helps to inhibit the growth of bacteria [41].

The total soluble solids indicated the moisture and sugar content in the honey. Generally, honey with high moisture and sugar content has a high TSS value. Thus, the TTS value indicates the water content of honey [42]. As for this study, the TTS value of honey is higher than the stingless bee honey which is 76.40. The brix value for stingless bee honey is low which ranged from 73.88 and 74.85.

3.3.2 Polyphenols in honey

Table 4: Phenolic profile and antioxidant properties of different types of honey [43]

Parameter	Tualang Honey (Multifloral)	Tualang forest honey (Multifloral)	Stingless bee honey (Monofloral)	Stingless bee honey (Multifloral)
Energy (kcal/100g)	316.66 ± 1.52	312 ± 2	275.3 ± 2.08	277.3 ± 2.1
Moisture (g/100 g)	24.8 ± 0.36	22.96 ± 0.89	29.8 ± 0.9	30.42 ± 0.68
Ash (g/100 g)	0.163 ± 0.052	0.386 ± 0.01	0.309 ± 0.01	0.293 ± 0.03
Carbohydrate (g/100 g)	77.66 ± 1.52	76.31 ± 0.41	68.1 ± 0.7	68.56 ± 0.76
Protein (g/100 g)	0.519 ± 0.03	0.686 ± 0.04	0.79 ± 0.01	0.75 ± 0.03
Fat (g/100 g)	0	0	0	0
Vitamin C (mg/100 g)	32.27 ± 1.7	26.72 ± 1.24	79.5 ± 0.65	87.19 ± 0.75
Total Phenolic content (mg GAE/kg)	139.42 ± 13.7	183.93 ± 24.1	228.09 ± 7.9	235.28 ± 0.6
Total Flavonoid content (mg CE/kg)	64.72 ± 11.4b	66.98 ± 7.32b	97.88 ± 10.1	101.5 ± 11.4

This study aims for the phenolic profile and antioxidant properties of different types of honey which covers the moisture, ash, protein, carbohydrate, fat, and energy. The data shows that mostly both stingless bee honey of different botanical origin have better properties than the Tualang honey. The vitamin C content in stingless bee honey is significantly higher than the Tualang honey. This also prove that sourness of stingless bee honey has been correlated with high vitamin C content.

The phenolic content of stingless bee honey also shows a big range of differences with both tualang honey with 228.09 ± 7.9 and 235.28 mg GAE/kg, respectively. The stingless bee honey of both types also shows the highest percentage of flavonoid content with 97.88 ±10.1 and 101.5 ±11.4, respectively.

Based on the study, it shows that there is a correlation between polyphenols content of honey and the antioxidant activity. Since polyphenol compounds continue to be a preventative agent in the scientists' theories against degenerative and chronic illnesses, its concentration of polyphenols is vital for proper therapeutic actions of honey since it is the most abundant phytochemical agents [43,44].

4. Conclusion

In this investigation, two techniques were developed to fulfil the aims of this study. Using a critical literature review method, bioactive components of sea cucumbers and stingless bee honey were studied. Furthermore, the full sequenced *Apostichopus japonicus* was employed in *silico* bioinformatics analysis to investigate target glycoproteins from sea cucumber. From this study, we succeeded in identifying bioactive compounds from sea cucumbers and stingless bee honey, most of which have experimental evidence to verify their stated beneficial activity for human benefit.

Bioinformatics analyses successfully created 3D protein models for 25 glycoproteins. Preliminary protein-ligand docking was done between glycoprotein models and their expected ligands. Due to lack of time, our early assumption of interaction between *Apostichopus japonicus* glycoproteins and stingless bee honey ligands was not tested. This bioinformatics data, however, will be helpful for further study into prospective uses of these glycoprotein targets in silico molecular dynamic modelling. The possibility for producing a novel functional meal combining sea cucumber extract with stingless bee honey and future synergy experiments to test this notion is also addressed. According to the results of a critical literature review and bioinformatics studies, sea cucumber and stingless bee honey are beneficial to human life and should be utilized sustainably. Experimental wet lab assays are strongly advised to evaluate this novel combination's synergy.

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