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FORMULATION AND EVALUATION OF GOLDEN SEA CUCUMBER (*STICHOPUS HERRMANNI*) ORAL SUSPENSION

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ABSTRACT

The Golden Sea Cucumber (*Stichopus herrmanni*) is widely recognized for its high protein and collagen content, making it valuable for medicinal applications, such as wound healing and tissue regeneration. The present study focused on formulating an oral suspension of sea cucumber extract, assessing its stability, and determining its protein content. Extraction was performed using a boiling method with distilled water to yield a protein-rich extract, which was then formulated into four suspension formulas (Formula 1, 2, 3, and 4) using pharmaceutical-grade excipients, including sorbitol, sodium CMC, and sodium benzoate. Protein quantification was conducted using the Biuret method with Bovine Serum Albumin (BSA) as a standard and analyzed using UV-Vis spectrophotometry. Stability testing over 1, 3, and 6 months was used to evaluate pH, viscosity, and sensory characteristics (odor, color, and taste). Among the four formulations, formula 4 demonstrated the highest stability, maintaining optimal homogeneity, sensory attributes, and pH, along with the highest protein concentration (8.10 mg/mL. These results highlight Formula 4 as the most promising because of its superior stability and protein content. This study underscores the challenges of long-term stability in protein-based suspensions, suggesting the need for further research into advanced excipient selection and encapsulation techniques to protect sensitive proteins. Overall, this research supports the potential use of marine-derived bioactive compounds in pharmaceutical applications, paving the way for sustainable healthcare and cosmetic products.

Keywords: golden sea cucumber, *Stichopus herrmanni*, oral suspension formulation, stability test

INTRODUCTION

Sea cucumbers are among the most abundant marine invertebrates, with over 1,400 species identified to date belonging to the order Holothuroidea. Their soft, elongated, and rounded bodies have led to the common name "sea cucumber" (Ramili *et al*., 2024; Widya *et al*., 2015). The golden sea cucumber (*Stichopus hermanii*) is one such species that is utilized in traditional medicine because of its substantial nutritional and medicinal value. Notably, golden sea cucumbers contain up to 82% protein, with 80% of this protein comprising of collagen (Ardiansyah, 2016). In general, sea cucumbers are recognized for their high protein content, making them a valuable source of nutrition and support tissue regeneration, particularly in wound healing processes. In simple terms, sea cucumbers contain approximately 59.8% protein, with variations in protein content ranging from 40.7-63% w/w under dry conditions and 2.5-13.8% w/w under wet conditions (Taurina and Andrie, 2023).

Proteins are essential macronutrients and are distinct from carbohydrates and fats, as they primarily contribute to structural formation in organisms rather than acting as the primary energy source (Bidara and Panita, 2023; Ejije *et al*., 2017). Proteins are integral to

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the formation of body cells and serve as an energy source when carbohydrate and fat reserves are depleted (Azhar, 2016). The protein content of golden sea cucumbers is typically extracted using solvents. The fundamental principle of extraction lies in separating compound components from the raw material with an appropriate solvent, which is carefully selected based on the chemical properties of the target compound (Rizki *et al*., 2022).

The extraction method employed in this study was maceration, in which the solvent type and concentration influenced the protein content of the resulting extract. Distilled water was chosen as the solvent because of its polarity, universality, and availability (Asworo and Widwiastuti 2023). Aromatic amino acids exhibit strong UV absorption, enabling proteins and peptides to absorb UV light in proportion to the concentration of aromatic amino acids present (Wang *et al*., 2022). Protein quantification can be carried out using several methods, including the Kjeldahl (Fauzi *et al*., 2023) method with titrimetric analysis and spectrophotometric techniques such as the Biuret (Zheng *et al*., 2017), Lowry (Mæhre *et al*., 2018), and Bradford methods (Kruger, 2009), which utilize UV-Vis spectroscopy for measurement. Protein quantification was conducted using the Biuret method coupled with spectrophotometry, with bovine serum albumin (BSA) serving as the protein standard. The Biuret method involves the conversion of the protein solution into a base with NaOH, which subsequently reacts with Cu^{2+} ions from $CuSO_4$ to form a blue-purple complex in an alkaline environment (Fauzi *et al*., 2022; Zheng *et al*., 2017).

Formulation is the initial step in pharmaceutical manufacturing, emphasizing the physicochemical properties of active compounds and their interactions with other components, which can significantly affect the stability, appearance, and overall formulation development (Wicita *et al*., 2021). The primary objective of this formulation is to provide critical data to developers, enabling the production of stable preparations with reproducible bioavailability at scale. Preformulation studies involve evaluating the physical and chemical properties of a compound to determine the optimal formula and dosage form (Dakrory *et al*., 2015). Thus, formulation encompasses the design and composition planning of active ingredients and excipients, informed by the preformulation study results (Sopyan *et al*., 2021).

This study investigated the formulation, preparation, and extraction of sea cucumbers using both wet (steaming) and dry processing methods. Additionally, an oral suspension formulation was developed in alignment with the guidelines of the Ministry of Health of the Republic of Indonesia (2015). An oral suspension is a liquid preparation that contains fine solid particles dispersed in a liquid medium (Hossain *et al*., 2022; Hossain *et al*., 2020). The suspension form was chosen over solid preparations due to its ease of swallowing, particularly for children and the elderly, who may have difficulty with solid dosage forms. Moreover, suspensions offer enhanced bioavailability and faster absorption than other dosage forms. This suspension was formulated to accommodate active drug substances that are water-insoluble, but require a liquid form for easier administration to patients (Kementerian Kesehatan RI, 2023).

RESEARCH METHODS

Golden Sea Cucumber (*Stichopus hermannii*) was sourced from Solo, Central Java, Indonesia. The extraction process utilized distilled water through boiling, along with $CuSO_4$, KNa Tartrate, and NaOH, all of analytical grade (Merck). The formulation materials included Sodium CMC, Potassium Sorbate, Citric Acid, Sodium Benzoate, and Sorbitol, all of pharmaceutical grade.

The equipment used was a heating stirrer (Thermo Scientific), UV-Vis spectrophotometer (Shimadzu UV-Mini 1280), pH meter (OHAUS), micropipettes of varying sizes (Socorex), laboratory glassware (Pyrex), Viscosimeter Brookfield, and an autoclave (GEA).

Research Procedure

Extraction of Sea Cucumber

One kilogram of Dried sea cucumber was placed in a heating pan containing 3 liters of water. Boiling was performed at 100°C using a heating stirrer for 3 hours until the sea cucumber became soft and easily disintegrated. The mixture was allowed to cool to room temperature before being filtered to remove all particulates. The extract was left undisturbed for 24 hours to allow any residual particles to settle, and the residue was filtered again using filter paper. The supernatant was collected in a glass container and sterilized by autoclaving at 121°C for 15 minutes.

Formulation of Sea Cucumber Extract Suspension

Distilled water (500 mL) was added to the beaker glass (1000 mL), and Na CMC, Na Benzoate, Citric Acid, and Potassium Sorbate, and the mixture was heated at 100 °C for 15 minute. Sorbitol was gradually added under continuous stirring until a homogeneous mixture was obtained. The sea cucumber extract was incorporated until the formulation was homogeneous. The variations in the formulation components are presented in Table 1.

Protein Content Determination (Biuret Method)

To prepare the biuret reagent, 1 g copper sulfate and 4 grams of potassium sodium tartrate were dissolved in 100 mL distilled water. Subsequently, 4 grams of sodium hydroxide was added and the volume was adjusted to 250 mL. Bovine Serum Albumin (BSA) standards were prepared at concentrations of 0.100, 0.200, 0.400, 0.600, and 0.800 µg/mL (Liu and Pan, 2017). For each standard or sample, 1 mL was mixed with 1 mL of Biuret reagent, and distilled water was added to reach a total volume of 5 mL. The protein content was measured using a UV-Vis spectrophotometer at the optimal wavelength and operating time, and the sample content was calculated based on a standard calibration curve.

Stability Test

The formulation was evaluated for stability over 1, 3, and 6 months, and stability was assessed based on appearance, color, odor, and taste. Further evaluations included pH measurement using a pH meter and viscosity measurement using a Brookfield viscometer.

RESULTS AND DISCUSSION

The Golden Sea Cucumber (*Stichopus herrmanni*) is a species of sea cucumber commonly found in Indonesian waters **Figure 1**. It is characterized by an elongated shape and golden-yellow color, with a length ranging between 2 and 30 cm.

Figure 1. Golden Sea Cucumber (*Stichopus herrmanni*) (Woo *et al*., 2015)

With its high protein and collagen content, this sea cucumber exhibits several biological activities, such as wound healing. However, if not stored in water, sea cucumbers quickly rot and become soft, making product processing essential for extending shelf life and improving usability. In this study, a suspension formula containing sea cucumber extract was developed, followed by stability testing and determination of protein content. The formulation of the golden sea cucumber oral suspension is listed in **Table I.**

Table I. Sea cucumber extract oral suspension formula

Component	Formula 1	Formula 2	Formula 3	Formula 4
Sea Cucumber Extract	50 mL	50 mL	50 mL	50 mL
Sorbitol	50 mL	150 mL	50 mL	150 mL
Sodium CMC	5 g	5 g	10 g	10 g
Citric Acid	2 g	2 g	2 g	2 g
Potassium Sorbate	1 g	1 g	1 g	1 g
Sodium Benzoate	1 g	1 g	2 g	2 g
Water (Aquadest)	Ad 1000 mL	Ad 1000 mL	Ad 1000 mL	Ad 1000 mL

Sea cucumbers were extracted using a simple boiling method in water. This approach was chosen because of its ease of protein solubility in water and the simplicity and feasibility of the process, which is particularly suitable for small traditional medicine industries (IKOT). Each formulation was evaluated for pH, viscosity, appearance, and sensory characteristics, and the results are shown in **Table II.**

Table II. Stability of Sea cucumber extract oral suspension

Formula	pH	Viscosity (cP)	Appearance	Odor	Taste
1	6.5	20	Clear	Fishy odor	Slightly sweet
2	4.5	30	Clear	Fishy odor	Slightly sweet
3	6.0	20	Clear	Fishy odor	Slightly sweet
4	3.8	30	Clear	Fishy odor	Slightly sweet

Stability is a crucial factor for pharmaceutical development. Stable products are not only safer for consumers but are also more effective in delivering health benefits. This study assessed the stability of the formula over periods of 1, 3, and 6 months. In the first month, formulas F1 and F2 showed a decrease in quality, as indicated by suspension turbidity and a rancid odor, likely due to suboptimal excipient combinations. One contributing factor to the decrease in quality was insufficient suspension viscosity. Adding Sodium CMC did not adequately increase the viscosity, and the preservative sodium benzoate was insufficient to inhibit bacterial growth, which caused spoilage. Sorbitol also influenced suspension viscosity because of its humectant properties, which help maintain suspension moisture. pH also impacted stability, especially regarding suspension integrity, with Formula 1 (F1) and Formula (F3) displaying pH values of 6.5 and 6.0, respectively.

Subsequent evaluations involved determining the protein content of both the sea cucumber extract and liquid sea cucumber preparation. The Biuret method, chosen for its simplicity, affordability, and speed, was used to measure the protein content in golden sea cucumber extract (Stichopus herrmanni). In this method, copper ions (Cu^{2+}) in a basic environment react with peptide bonds in proteins to form a blue-purple complex. This color was measured using a UV-Vis spectrophotometer at a wavelength of 550 nm, with higher protein concentrations producing more intense coloration. Bovine Serum Albumin (BSA) was used as a protein standard, with concentrations ranging from 100 to 800 µg/mL, providing a clear basis for calculating the protein content in the sea cucumber extract. Spectrophotometric measurements were calibrated to determine the protein concentration in each tested sample.

Table III. Results of Protein Content Determination for Each Oral Suspension Formula

	Formula 1	Formula 2	Formula 3	Formula 4
Protein Concentration (mg/mL)	7.99±0.02	7.27±0.08	7.35±0.05	8.20±0.10

This study explored multiple formula variations to examine how excipient modifications impacted measurable protein levels **Table III.** Results showed notable differences, with protein content across formulas ranging from 7.25 mg/mL to 8.10 mg/mL, with Formula 4 yielding the highest protein content. Excipients such as sorbitol, which functions as both a sweetener and stabilizer, significantly improved the solution homogeneity. Sodium CMC, acting as a thickening agent, played a crucial role in ensuring that the protein particles remained well dispersed in the suspension. Formula 4, which combined sorbitol and sodium CMC concentrations more optimally, demonstrated increased stability and higher protein content. A statistical analysis was conducted using a licensed version of Microsoft Excel to evaluate the differences in protein content across four different formulations (Formula 1, Formula 2, Formula 3, and Formula 4). The results of the one-way ANOVA revealed a statistically significant difference in protein levels among the groups, with an F-value of 137.587 and a significance level (p-value) of 0.000. As the p-value is less than 0.05, it indicates that the variation in protein content is significantly affected by the type of formulation used. The between-group sum of squares (1.933, df = 3) was considerably higher than the within-group sum of squares (0.037, df = 8), suggesting that the observed differences were due to formulation differences rather than random variation. These findings demonstrate that the formulation had a significant impact on protein concentration.

The high protein content of golden sea cucumber holds substantial potential for pharmaceutical and cosmetic product development, especially for tissue regeneration and skin rejuvenation applications. The accurate determination of protein levels in sea cucumber-based products is essential to ensure therapeutic efficacy and dosage consistency. The choice of oral suspension is intended to facilitate administration, particularly in pediatric and elderly populations. Oral suspensions also offer improved bioavailability, allowing active compounds to be absorbed more quickly. Oral suspensions provide superior bioavailability, enabling faster absorption of active compounds than solid dosage forms. This study also underscores the challenges of ensuring long-term stability of protein-based suspensions, as exemplified by the stability limitations observed in Formulas 1 and 2.

The formulation of stable pharmaceutical suspensions is a complex process, particularly for protein-based formulations. This study highlights the need for additional research to optimize excipient selection, with a particular emphasis on thickeners and preservatives. Advanced techniques, such as microencapsulation and nanoencapsulation, can be used to enhance stability by shielding protein molecules from environmental stressors. Clinical trials are also essential to confirm the safety and efficacy of these formulations, ensuring compliance with regulatory standards and aligning with consumer expectations for reliable and effective products.

In conclusion, this study offers valuable insights into the formulation and stabilization of suspensions of golden sea cucumber extract. These findings contribute to the advancement of marine-derived pharmaceutical products with diverse applications in healthcare and cosmetic industries, supporting the continued exploration of bioactive compounds from marine biodiversity.

CONCLUSION

In this study, we developed an oral suspension of golden sea cucumber (Stichopus herrmanni) extract, highlighting its wound healing potential. Formula 4 showed the best stability over six months, maintaining key attributes and the highest protein concentration (8.10 mg/mL). Although promising, challenges in long-term protein stability warrant further research. This study supports the use of marine bioactive compounds in pharmaceuticals and cosmetics.

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