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Application of Solid Lipid Nanoparticles Method in Black Sea Cucumber (*Holothuria atra)* Extract Peel-off Gel Mask and Antioxidant Activity Tests

(Penerapan Metode Nanopartikel Lipid Padat pada Masker Gel *Peel-off* Ekstrak Teripang Hitam (*Holothuria atra*) dan Uji Aktivitas Antioksidan)

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Abstract: Black sea cucumbers (*Holothuria atra*) are a potential source of active metabolites such as flavonoids widely used for nutraceutical, pharmaceutical, and cosmetic products. The type of nanoparticle that is often used in cosmetics and skin products is Solid Lipid Nanoparticles (SLN). This research aimed to optimize the use of black sea cucumbers as Indonesia's natural resources to be formulated into a topical antioxidant peel-off gel mask dosage form that meets the physical and chemical requirements. Black sea cucumbers were extracted using the maceration method, while SLN preparation used high-shear homogenization and freeze-drying methods. Crude extract and SLN were formulated into peel-off gels and then evaluated, including organoleptic, homogeneity, viscosity, pH, spreadability, drying time, tensile strength test, and antioxidant activity using the DPPH method. The IC_{50} values of blank (F1), peel-off gel extract (F2), and nanoparticle peel-off gel extract (F3) were each 600.8727±0.43; 580.7849±0.7; and 592.76±0.55 µg/mL. In conclusion, the SLN method can be applied to the formulation of black sea cucumber extract nanoparticles. However, peel-off gel masks from crude and nanoparticle extracts have very weak antioxidant activity.

Keywords: Antioxidants, *Holothuria atra*, solid lipid nanoparticles, black sea cucumber

Abstrak: Teripang hitam (*Holothuria atra*) merupakan sumber potensial metabolit aktif seperti flavonoid yang banyak digunakan untuk produk nutrasetikal, farmasi, maupun kosmetik. Nanopartikel yang sering digunakan dalam produk kosmetik untuk kulit adalah *Solid Lipid Nanoparticles* (SLN). Penelitian ini bertujuan untuk mengoptimalkan pemanfaatan teripang hitam sebagai sumber daya alam Indonesia untuk diformulasikan menjadi bentuk sediaan masker gel peel-off antioksidan topikal yang memenuhi persyaratan fisika dan kimia. Teripang hitam diekstraksi menggunakan metode maserasi, sedangkan formulasi SLN menggunakan metode *high shear homogenization* dan *freeze drying*. Ekstrak dan SLN diformulasikan menjadi gel *peel-off*, kemudian dievaluasi meliputi organoleptik, homogenitas, viskositas, pH, daya sebar, waktu pengeringan, uji kekuatan tarikan, dan aktivitas antioksidan dengan metode DPPH. Nilai IC_{50} blanko (F1), gel *peel-off* ekstrak (F2), dan gel *peel-off* nanopartikel (F3) masing-masing sebesar 600,8727±0,43; 580,7849±0,7; dan 592,76±0,55 µg/mL. Dapat disimpulkan metode SLN dapat diterapkan pada formulasi nanopartikel ekstrak teripang hitam namun masker gel peel-off dari ekstrak dan nanopartikel ekstrak memiliki aktivitas antioksidan yang sangat lemah.

Kata kunci: Antioksidan*, Holothuria atra,* solid lipid nanopartikel, teripang hitam

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INTRODUCTION

SEA CUCUMBERS are marine invertebrates with bioactive compounds as an adaptation strategy in extreme marine environmental conditions and a protection strategy to escape predators[1]. Typical compounds sea cucumbers possess are triterpene glycosides, saponins, and flavonoids as secondary metabolites. Flavonoids produced by sea cucumbers are supposed to have biological effects, including antifungal, antioxidant, and anticancer properties[2]. Black sea cucumbers also contain collagen, one of compounds having anti-aging properties[3]. Previous studies have shown that the sea cucumber species *Holothuria atra* has a reasonably high antioxidant activity among other types of sea cucumbers from the genus *Holothuria* that were studied[4].

Black sea cucumber extract is formulated in form of nanoparticles to increase its penetration into skin barrier. The application of nanotechnology in the pharmaceutical field has many advantages, including increasing the solubility of compounds, reducing medication doses and increasing absorption. Therefore, nanoparticle materials are widely used in cosmetic and dermatological preparations[5]. The type of nanoparticle that is often used in cosmetics and skin products is Solid Lipid Nanoparticles (SLN). SLNs are developed from existing conventional carriers, such as emulsions, liposomes and polymer nanoparticles, such as colloidal barriers to control drug delivery[6].

In this study, the SLN of black sea cucumber extract were prepared in the form of a peel-off gel mask as an antioxidant. This gel preparation used a mixed gelling agent from polyvinyl alcohol (PVA) with hydroxypropyl cellulose medium (HPC-m). Polyvinyl alcohol was chosen as the film former because it can produce a gel that dries quickly and forms a strong, plastic, and thin film that is easy to remove after drying and can provide good contact between the skin and the active substance[7]. This research aimed to optimize the use of black sea cucumbers as Indonesia's natural resources to be formulated into a topical antioxidant peel-off gel mask dosage form that meets the physical and chemical requirements.

MATERIALS AND METHODS

MATERIAL. Black sea cucumber (*Holothuria atra*) (Determined at LIPI Oceanography, Ancol, North Jakarta, Indonesia), stearic acid (Merck Chemicals and Life Sciences, Jakarta, Indonesia), Tween 80 (Brataco, Jakarta, Indonesia), PEG 400 (Merck Chemicals and Life Sciences, Jakarta, Indonesia), 96% ethanol (Mallinckrodt Pharmaceuticals, America), lactose (Merck Chemicals and Life Sciences, Jakarta, Indonesia), polyvinyl alcohol (Brataco, Jakarta, Indonesia), propylene glycol (Brataco, Jakarta, Indonesia), hydroxy propyl cellulose-medium (Nippon Soda Co. LTD, Tokyo, Japan), sodium benzoate (DSM, Netherlands), sodium metabisulfite (Brataco, Jakarta, Indonesia), sodium laureth sulfate (BASF Distribution Indonesia), disodium EDTA (Brataco, Jakarta, Indonesia), Pure water.

Tools. Analytical balance type AD-300H (ACIS, Jakarta, Indonesia), pH meter PHM201 (Meterlab, Daejeon, Republic of Korea), Viscometer Brookfield DV-II+ Pro (Toronto, Canada), laboratory glassware (Pyrex, Staffordshire, United Kingdom), stirrer (IKA Eurostar, Staufen im Breisgau, Germany), Homogenizer Ultra Turax T25 Digital (IKA, Staufen im Breisgau, Germany), water bath W 600 (Memmert, Büchenbach, Germany), UV-Vis spectrophotometer UV-1601 (Shimadzu, Kyoto, Japan), glass slides and cover glasses, tools for measuring gel dispersion ability (glass, 200 g weights, Teflon rings, and vernier callipers), freeze dryer, zeta potential analyzer (Malvern, United Kingdom), Scanning Electron Microscopy (JSM6400; JEOL, Tokyo, Japan), Strograph-R1 (Toyo Seiki, Japan).

METHODS. Preparation of Black Sea Cucumber Ethanol Extract[8]. Dried samples from black sea cucumbers were weighed as much as 500 g, put into a maceration vessel, and a 96% ethanol solvent solution was added. The ratio of the sample to the solvent is ten parts to 75 parts of the solvent. The maceration vessel was then tightly closed and left for 24 hours while stirring and stored in a place that was not exposed to sunlight. After 24 hours, the solution was filtered using filter paper and the dregs were macerated again by adding solvent. Maceration was stopped if the liquid or solvent was no longer coloured. The extracts obtained were then collected and concentrated using a rotary evaporator. Characterization of the black sea cucumber ethanol extract included organoleptic, pH, phytochemical screening of flavonoids using Wilstater reagent, and protein screening using the Biuret method[9,10].

Preparation of Black Sea Cucumber Ethanol Extract Solid Lipid Nanoparticles. Preparation of SLNs was using the high shear homogenization and freeze-drying methods[11]. The lipid phase was carried out by melting stearic acid (1% w/v) at 75 °C. Ethanol extract of black sea cucumber (1% w/v), ethanol (10% w/v), and propylene glycol (20%) were added into the lipid phase. Preparation of the aqueous phase was carried out by dissolving Tween 80 (20% w/v) and PEG 400 (20% w/v) in distilled water (28%

w/v) then heating it to the melting temperature of the lipids. The surfactant solution was then added to the lipid phase, heated to a temperature close to 75 °C and then stirred using a homogenizer at a speed of 24,000 rpm for 10 minutes. The emulsion formed was then dispersed in cold distilled water (4 °C) containing 8% lactose, with a ratio of 1:1, while homogenizing at 5000 rpm for 5 minutes to cool. The dispersion was then stored in the refrigerator at 4 °C. Before the freeze-drying process, the SLN dispersion was frozen in a refrigerator at 2 °C and then lyophilized for 72 hours at -80 °C.

Characterization of SLN Containing Black Sea Cucumber Ethanol Extract[11]. Determination of particle size and polydispersity index (PDI) was done by dispersing SLN in distilled water with a ratio of 1:15 (v/v) at 25 °C. Measurements were made using a Particle Size Analyzer. Examination of zeta potential was using a zeta potential analyzer at 25 °C and analysis of particle morphology was using Scanning Electron Microscopy (SEM).

Formulation of Black Sea Cucumber Extract Peel-off Gel Mask. The formula used was shown in Table 1. HPC-m was first developed with 96% ethanol, then allowed to stand for 24 hours, and homogenized using a stirrer. Polyvinyl alcohol (PVA) was added to pure water at room temperature and then heated over a water bath at 80 °C until it expands, cooled and then homogenized using a stirrer. HPC-m and PVA were then mixed until homogeneous with propylene glycol. Sodium metabisulfite and disodium EDTA were dissolved in pure water and then added to the ethanol extract of black sea cucumbers. Sodium benzoate and sodium laureth sulfate were dissolved in water, respectively. The extract solution, Na benzoate, and Na laureth sulfate were put into the HPC-m and PVA mixture and then homogenized using a stirrer at 1500 rpm for 30 minutes.

Evaluation of the Peel-off Gel Mask[12]. Masks were evaluated organoleptic, including colour, odour, and shape. Homogeneity test was carried out by smearing the preparation on a glass object and observing its homogeneity using a microscope. Viscosity test was evaluated using a Brookfield DV-II+ Pro viscometer. pH test was evaluated using a pH meter. Spreadability test was carried out by placing the emulgel on a glass scale, coated with glass, and given a load of 50 grams. The spreading diameter was measured when the preparation stopped spreading. Drying time was measured by applying 50 mg of peel off mask gel preparation was applied to the inner skin of the upper arm with an area of 5 cm x 5 cm, then the speed of drying was measured to form a peel off layer of gel using a stopwatch. Tensile strength was measured using tensile strength tester.

Peel-off Gel Mask Antioxidant Activity Test[13]. A total of 25 mg of the sample was diluted with methanol pro-analysis to obtain a concentrated solution of 1000 µg/mL. Pipette the solution as much as 5.0 mL to get a concentration of 400; 500; 600; 700; and 800 µg/mL. Pipette 4.7 mL of each concentration into a test tube and then add 0.3 mL of 160 ppm DPPH solution. The mouth of the tube was covered with aluminium foil and then homogenized. DPPH solution without inhibition (blank solution), test solution, and positive control solution of vitamin C (0.25, 0.5, 1, 2, 4 µg/mL) were immediately incubate for 10 minutes at room temperature ±25 °C (protected from light), then measure the absorbance at a wavelength of 516 nm. The resulting data was calculated using the regression line equation obtained from the curve of the relationship between concentration as the x-axis and the scavenging value of free radicals as the y-axis. Inhibition of free radical activity[14] (%) =

$$\frac{A\ blank - A\ sample}{A\ blank}\ x\ 100\%$$

Table 1. Black sea cucumber extracts peel-off gel mask formula.

Ingredients	Formula		
	F1	F2	F3
Holothuria atra Extract (crude)	-	0.16	-
Holothuria atra Extract (SLN)	-	-	0.16
Polyvinyl Alcohol	10	10	10
Propylene Glycol	10	10	10
Hydroxy Propyl Cellulose-medium	2	2	2
Sodium Benzoate	0.2	0.2	0.2
Sodium Metabisulfite	0.1	0.1	0.1
Sodium Laureth Sulfate	1	1	1
Disodium EDTA	0.1	0.1	0,1
Perfume	q.s	q.s	q.s
Ethanol 96%	10	10	10
Water up to	100	100	100

RESULTS AND DISCUSSION

Preparation of Black Sea Cucumber Ethanol Extract. The black sea cucumber was determined at LIPI Oceanography, Ancol, North Jakarta, with number B-807/IPK.2/LT.01/III/2016. Dried black sea cucumber (*Holothuria atra*) extract was prepared using 96% ethanol solvent by repeated maceration. The results of the organoleptic examination of the ethanol extract of black sea cucumber following previous studies, which have a solid form, like a yellow-brown to dark brown paste, with a characteristic fishy odour[15].

The black sea cucumber ethanol extract has a pH of 5.41±0.02. This result could be due to the content of flavonoid compounds in the extract, which are bound to sugars, ethers and also phenolic acids, which tend to be weak acids[16]. These results were supported by examining the flavonoid content of the black sea cucumber ethanol extract, which gave positive results as indicated by a reddish-orange colour on the amyl alcohol layer.

The results of examining the protein content of black sea cucumbers in previous studies showed negative effects in n-hexane, ethyl acetate, and methanol extracts[17]. However, the results of this study gave positive results for the ethanol extract, which was marked by a change in colour to purple (Figure 1). Copper (II) ions and peptide bonds will form a purple complex in alkaline solution, the more peptides that bind to Cu^{2+} ions, the more intense the purple color will be. Black sea cucumber contains 80% collagen[18]. Collagen is a fibrous protein and is the main component of connective tissue found in many marine resources[19].

Preparation of Black Sea Cucumber Ethanol Extract SLN. In the manufacture of SLN, the liquid lipid phase containing the extract is mixed with the surfactant solution phase. This mixture was heated at a temperature of 5-10 ºC above the lipid's melting point, accompanied by mechanical stirring at high speed so that friction occurs between the particles, which will cause a reduction in particle size[20]. The formation of SLN was indicated by the appearance of colloid and the change in colour to a cloudy white, slightly bluish translucent with a weak, fat aroma. The use of ethanol serves to increase solubility so that the extract can be adequately absorbed in stearic acid. Tween 80 acts as an emulsifier to reduce the interfacial tension of the lipid phase so that it can mix with the aqueous phase by forming a layer that coats the oil phase. PEG 400 and propylene glycol act as co-emulsifiers which help stabilize the formed emulsifier layer, thereby increasing the stability of the SLN. Propylene glycol can prevent ethanol evaporation and increase the solubility of the extract. Lactose is used as a cryoprotectant to protect the SLN system from particle aggregation and coalescence during freeze-drying. Lactose acts as a cryoprotectant because lactose can form a protective shell layer around the SLN as a hydrogen bond between the –OH group of lactose and the -COO- group resulting from the hydrolysis of carboxylic acids from the lipid matrix[21].

Characterization of SLN Containing Black Sea Cucumber Ethanol Extract. The results of the SLN characterization of black sea cucumber extract can be seen in Table 2. SLN has an average particle size of 63.65±1.7 nm. This result has met the requirements, where a particle was considered nano-sized if it is 1-1000 nm. The SLN of black sea cucumber extract

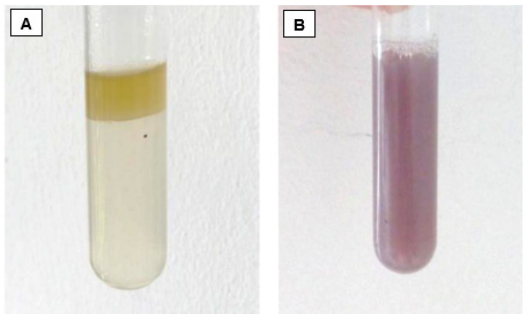


Figure 1. Screening results of black sea cucumber extract on A) flavonoid and B) protein.

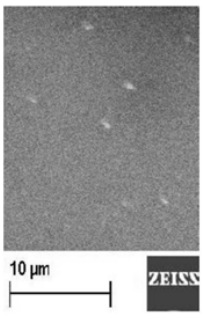


Figure 2. Morphology of SLN containing black sea cucumber ethanol extract.

Table 2. Characterization results of SLN containing black sea cucumber ethanol extract.

Replication	Particle size (nm)	Polydispersity index (PDI)	Zeta potential (mV)
I	63.19	0.339	-4.78
II	62.23	0.332	-2.53
III	65.53	0.290	-3.95
Average	63.65±1.70	0.320±0.030	-3.75±1.14

The result was presented in mean ± SD, n=3.

Table 3. Evaluation results for peel-off gel mask preparations.

Evaluation	Formula		
	F1	F2	F3
Colour	colourless	clear yellow	white
Odour	flower scent	flower scent	flower scent
Texture	semisolid	semisolid	semisolid
homogeneity	homogeneous	homogeneous	homogeneous
Viscosity (cps)	5215	4741	4492
Dry time (minutes)	36.19±1.49	25.95±1.97	27.44±2.3
Spreadability (cm^2)	4.59	5.65	6.25
Tensile strength (kg/cm^2)	84.66±3.33	97.15±5.3	78.01±3.49
pH	6.43±0.01	5.5±0.01	5.71±0.01

The result was presented in mean ± SD, n=3.

has a polydispersity index value between 0.320±0.03, indicating that the SLN of black sea cucumber extract is homogeneously dispersed. The results of this characterization and polydispersity index suggest that the speed and duration of stirring during manufacture are optimal.

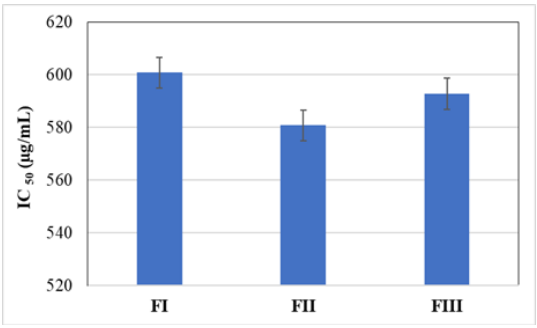
Based on the results of the examination, it was found that the zeta potential value was -3.75±1.14 mV. The greater the zeta potential value of ±25 mV, the more stable the colloidal nanoparticles will be. The morphology of nanoparticles is shaped like a prism and is larger due to the accumulation of particles (Figure 2). This aggregation can be caused by a zeta potential value close to neutral; this can be caused by tween 80, which is used as a surfactant and works on the outer layer of the nanoparticles, which has no charge or is nonionic. The negative value obtained comes from the use of stearic acid[11].

Peel-off Gel Mask Preparation Formulation. Observation and evaluation of gel peel-off mask preparations were carried out on three formulas, F1, F2 and F3. The difference between the three formulas was found in the active ingredients used, namely F1 (blank, without active ingredients), F2 (crude black sea cucumber extract), and F3 (SLN black sea cucumber extract). The results of the preparation evaluation in Table 3 showed that the three formulas have met the desired physical and chemical quality parameters. The colours of the three formulas are different because they depend on the active ingredients added, but for the shape and aroma, all three have a floral scent from the added perfume and have a semisolid form. All three formulas produce homogeneous preparations to provide the same therapeutic effect for each use.

Based on observing the viscosity of the extract peel-off gel and the black sea cucumber extract nanoparticle peel-off gel at 60 rpm on the Brookfield DV-II+ Pro viscometer, the peel-off gel blank had the greatest viscosity. This result was caused by no extract or nanoparticles in the blank, which can reduce the viscosity of the gel. The lower viscosity of the extract peel-off gel and black sea cucumber extract nanoparticles resulted in increased dispersion ability when given the same pressure[22]. The spreadability between blank with F2 and F3 had a significant difference ($p<0.05$), while there was no significant difference in the ability to spread F2 and F3.

The results of the drying speed test showed that the peel-off gel preparations made from PVA–HPCm had a drying time range of 25.95–36.19 minutes. Where F1 requires the longest drying time, this can result from the high viscosity of the blank so that when drying, the peel-off layer takes longer to dry completely[23]. There was no significant difference in the drying time of F2 and F3, so it is known that they will form a short dry peel-off layer.

The greater the tensile strength of the peel-off gel, the better the gel will be, and the dried peel-off layer has elastic properties and is not easily brittle. The characteristics of the peel-off gel, which was elastic and not easily brittle, were due to the use of the PVA-HPCm polymer combination. PVA provides elastic properties, and HPCm can produce clear gels. So that when consumers use it, it will not be challenging to remove the peel-off layer from the skin. The test results showed that F1 did not experience a significant difference from F2 and F3, so it can be said that the active ingredients used did not affect the tensile strength of the preparation ($p>0.05$). Based on observations, peel-off gel masks have a pH of 5.49–6.44; this pH



The result was presented in mean ± SD, n=3.

Figure 3. The results of peel-off gel antioxidant activity test.

value meets the requirements because it is included in the pH range on the skin surface, namely 4.5–6.5. Topical preparations that are too acidic or alkaline can damage the skin and cause skin irritation[24].

Peel-off Gel Mask Antioxidant Activity Test. Based on the results of testing the antioxidant potential of the preparations (Figure 3), the IC_{50} values of blank (F1), peel-off gel extract (F2), and nanoparticle peel-off gel extract (F3) were each 600.8727±0.43; 580.7849±0.7; and 592.76±0.55 µg/mL. In the blank formula, the antioxidant activity was produced from the antioxidant substances used as a composition in the blank formula. After adding extracts and SLN extracts, the IC_{50} value of the peel-off mask gel decreased, indicating the presence of ingredients antioxidants added. Statistically, it showed that F2 and F3 between F1 had significantly different antioxidant abilities ($p<0.05$), but the antioxidant abilities of these three formulas were still in the very weak category. Black sea cucumber extract in another study was found to have potential as a source of antioxidants with an IC_{50} value of 126.19 µg/mL[25]; however, in other studies, it showed a very weak antioxidant value[26,27]. This result can be caused by the degeneration of antioxidant compounds in the gel-making process caused by several factors, such as temperature. Based on previous research, the IC_{50} value of black sea cucumber extract decreased by more than 50% at 90 ºC for 30 minutes, and the flavonoid value decreased by more than 50% at 60 ºC for 90 minutes[28].

CONCLUSION

The solid lipid nanoparticle method can be applied to the formulation of black sea cucumber extract nanoparticles. Peel-off gel masks from the crude extract and nanoparticles (SLN) extract have met the physical and chemical requirements but have very weak antioxidant activity.

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