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(RESEARCH ARTICLE)



Formulation and Evaluation of the sea cucumber, *Holothuria arenicola* extract incorporated skin cream

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Abstract

Background: Cucumber extract is often used for skin problems, wrinkles, sunburn, and as an antioxidant. Thereby, the main objective of the present investigation was to develop a topical skincare cream (w/o) loaded with the sea cucumber, *Holothuria arenicola* extract (HaE).

Methods: Oil in water emulsion-based cream was prepared by incorporating liquid paraffin, soft wax, cetostearyl alcohol in the oil phase, and borax in the water phase respectively with 1 gm of HaE. The evaluation of HaE cream was done on different parameters like ph, viscosity, spreadability, extrudability, irritancy and stability.

Results: HaE showed good appearance, homogeneity, spreadability, diffusion, consistency, pH, extrudability, and spreadability without any irritancy to skin. The present results showed that HaE cream has radical scavenging activity in range from 57 to 70 % and NO scavenging activity in range from 25 to 49 %. **Conclusion:** The formulated HaE cream was successfully developed that met the relevant pharmaceutical characteristics form. The developed HaE cream could be added in topical formulations in order to protect skin against damage caused by free radicals due to its antioxidant activity.

Keywords: *Holothuria arenicola*; Antioxidant; Cream; skin

1. Introduction

Cosmeceuticals are cosmetic-pharmaceutical hybrids, containing active ingredients in creams, lotions, and ointments [1]. Semisolids constitute a significant proportion of pharmaceutical dosage forms intended to produce desired therapeutic action. Creams are semisolid dosage forms that contain one or more drug substances deliberated for application to the skin or mucous membrane. Emulsions are a class of dispersed systems consisting of two immiscible liquids. Oil in Water (O/W) emulsions are the most commonly used cosmetic delivery systems that supply moisture to the skin and improve the skin condition by forming the occlusive barrier on the skin.

In recent years, extensive studies have been conducted to explore biological activities, nutritional value, and potential health benefits of marine-based bioactive substances. [2,3] Natural formulations of cosmetics have a growing demand in the world market and are an invaluable gift of nature. These formulations always have attracted considerable attention because of their good activity and comparatively lesser or nil side effects with synthetic drugs. Thus, the cosmetic industry is seeking alternative products of natural origin and avoiding the use of synthetic ingredients [4]. Marine resources represent an underexploited source of highly diverse valuable compounds [5] with potential

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applications for the formulation of nutraceuticals and cosmetics [6]. Sea cucumbers are marine animals from the class *Holothuroidea* and have been conventionally employed as medicines owing to their therapeutic efficacy in various diseases. Several studies have already reported multi-faceted biological activities of the sea cucumbers species, the antioxidant, wound healing, and anti-ulcerogenic potential among the most commonly reported. More recently, it was rationalized that a formulation containing sea cucumbers extracts would be highly beneficial in cosmetic industries [7]. Cucumber extract is often used for skin problems, wrinkles, sunburn and as an antioxidant. The most important and abundant sea cucumber species in the Mediterranean Sea on the Egyptian coast is *Holothuria arenicola*.

Oxidative stress plays an important role in skin aging [8,9]. Antioxidant molecules slow the process of aging either by preventing free radicals from oxidizing sensitive biological molecules or by reducing the formation of free radicals and quenching the already formed reactive oxygen species [ROS] [10]. Consequently, cosmetology has a significant role in the prevention and attenuation of skin aging through the formulation of new substances with effective antioxidant activity, to be incorporated on cosmetic products for daily care [11]. Topical supplementation with antioxidants is regarded as a useful strategy that may improve skin antioxidant capacity to reduce ROS induced skin damages [12]. The antioxidant activity of the sea cucumber, *Holothuria arenicola* has been evident and up to date, there is no cosmetics formulation for such sea cucumber in Egypt. Thereby, the main objective of the present investigation was to develop a topical skincare cream [w/o] loaded with the sea cucumber, *Holothuria arenicola* extract [HaE].

2. Material and methods

2.1. Sample collection and preparation

Sea cucumbers (*Holothuria arenicola*) were collected from Abu-Qir Bay in the Egyptian Mediterranean coast at the eastern Alexandrian coast (May-June 2020). The animals were transported to our laboratory in an icebox containing ice cubes and a few pinches of table salt. The animals were immediately washed under running tap water and cut open, and all visceral organs were removed and then the body walls of the animals were stored at -20°C until processing. The phosphate buffer extract was prepared according to the method of Yasumoto et al. [13]. The body wall of the animals was cut into small parts and blended in phosphate buffer (in a volume = 4 ml × tissue weight) and extracted at room temperature (25°C) with pH 7.2 for 5 hours, the filtered was collected. The collected, filtered off the Sea cucumbers concentrated and lyophilized using a lyophilizer (LABCONCO, shell freeze system, USA).

2.2. Formulation of animal Ointments

Oil in water emulsion-based cream was prepared by incorporating liquid paraffin, soft wax, cetostearyl alcohol in the oil phase and borax in the water phase respectively. Both the oil and aqueous phases were heated to 75°C. After heating, the water phase was added into the oil phase with continuous stirring until homogenous cream was formed. After complete emulsification, HaE (1 gm) was added when the temperature dropped to 55 °C ± 60 °C. (Table 1)

Table 1 Cream constituents

Constituents	Quantity
Liquid Paraffin	30 gm
Soft Wax	9 gm
Cetostearyl Alcohol	2 gm
Borax	0.6 gm
Water	20 ml

2.3. Physical properties of the cream

2.3.1. Color, odor, and consistency

The color, odor, and consistency of the formulated ointments were examined visually.

2.3.2. Determination of pH

5 ± 0.01g of the Cream was weighed accurately in a 100ml beaker. 45ml of water was added & dispersed the Cream in it. The pH of the suspension was determined at 27o C using the pH meter.

2.3.3. Diffusion study

The diffusion study was carried out by preparing agar nutrient medium of any concentration. It was poured into petri dish. A hole bored at the center and ointment was placed in it. After 1 hr the distance that the ointments get diffused was measured [14].

2.3.4. Viscosity

Brookfield digital viscometer was used to measure the viscosity (in cps) of the prepared ointment formulations as such that is in a semisolid state [15].

2.3.5. Spreadability

It is the most desirable property of an ointment that decides the extent of the area to which the cream readily spreads on application to the skin or affected part. The spreadability was expressed in seconds taken by two slides to slip off from ointment placed in between the slides under the direction of a certain load. The lesser the time is taken for the separation of two slides, the better the spreadability [16]. Spread ability was calculated by using the formula.

$$S = (M.L/T)$$

Where, S = Spread ability,

M = Weight tied to upper slide,

L = Length of glass slides and

T = Time taken to separate the slides

2.3.6. Loss on drying (LOD)

LOD was determined by placing the formulation in petri-dish on a water bath and dried for the temperature 105°C.

2.3.7. Phase separation study for Emulsion bases

The ointments formulated using emulsion bases were evaluated for the signs of phase separation on Centrifugation at low, medium, and high speeds. A specific quantity of ointment was filled into the centrifuge tube and kept in the centrifuge. Then it was subjected to rotations at low speed for 5 mins followed by medium speed for 5 mins and high speed for 5 mins and any signs of separation of phases and change in consistency were checked [17].

2.3.8. Test for Thermal Stability

Thermal stability of the formulation was determined by the humidity chamber controlled at 60- 70% RH and $37 \pm 1^\circ\text{C}$

2.3.9. Stability studies

Stability testing of drug products begins as a part of drug discovery and ends with the demise of the compound or commercial product. To assess the drug and formulation stability, stability studies were done according to ICH guidelines. The stability studies were carried out as per ICH guidelines. The cream-filled in a bottle and kept in the humidity chamber maintained at $30 \pm 2^\circ\text{C} / 65 \pm 5\% \text{ RH}$ and $40 \pm 2^\circ\text{C} / 75 \pm 5\% \text{ RH}$ for two months. At the end of the studies, samples were analyzed for the physical properties and viscosity [18].

2.3.10. Tube extrudability

In the present study, the method adopted for evaluating cream formulation for extrude ability was based upon the quantity in percentage cream extruded from a tube on the application of finger pressure. More quantity extruded better was extrude ability. The formulation under study was filled in a clean, lacquered aluminum collapsible 5 grams tube with a nasal tip of 5mm opening and apply the pressure on the tube with the help of a finger. Tube extrude ability was then determined by measuring the amount of cream extruded through the tip when pressure was applied on the tube.

2.4. Irritancy test

Mark an area (1sq.cm) on the left-hand dorsal surface. The cream was applied to the specified area and time was noted. Irritancy, erythema, edema, was checked if any for regular intervals up to 24 hrs and reported [19].

2.5. Free radical scavenging activity

The free radical scavenging activity of HaE cream was analyzed by the DPPH assay [20]. A 1.0 ml of the cream, at gradient final concentrations of 10 - 50 mg/ml, was mixed with 2 ml of 0.3 mM DPPH solution in MeOH in a cuvette. The

absorbance was taken at 517 nm after 20 minutes of incubation in the dark at room temperature. The experiment was done in triplicates. The percentage free radical scavenging activity was calculated as follows:

Where, RSA is the Radical Scavenging Activity;
Abs control is the absorbance of DPPH radical + ethanol;
Abs sample is the absorbance of DPPH radical + extract.

2.6. Nitric oxide scavenging assay

The nitric oxide scavenging activity of HaE cream was analyzed by the Govindarajan et al. assay [21]. A 1.0 ml of the cream, at gradient final concentrations of 10 - 50 mg/ml, was mixed with 0.5 ml of sodium nitroprusside (5mM) and incubate for 30 min. Then 1.5ml of Griess reagent was added to each test tube. The absorbance was measured, immediately, at 546 nm. The percentage of nitric oxide scavenging activity was calculated as follows.

$$\% \text{ RSA} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where, RSA is the nitric oxide scavenging activity;
Abs control is the absorbance of tube test without extract;
Abs sample is the absorbance of tube test with extract

3. Results

- Physical properties
- Physical properties of HaE represented in table 2.
- Appearance and odor: HaE the cream was white-yellowish semisolid cream without odor.
- Consistency and feeling: HaE cream was smooth and cold cream.
- Loss on drying: LOD was 15.4 % at temperature 105 °C.
- pH: The pH of the cream was found to be in the range of 7 which is good for skin pH.
- Viscosity, spreadability, and diffusion: The viscosity of cream was 25240 cps which indicates that the cream is easily spreadable by 38 g cm/sec and easily diffuse by 0.3 cm.
- Phase separation: recorded at medium and high speed.
- Density and Solubility; Density of HaE cream was 0.89 (g/cm³) while the cream was insoluble in alcohol, ether, DMSO, acetone, and soluble in xyole.
- Thermal Stability: cream was thermally stable at 37o,25o, and 2oc. There was no phase separation.
- HaE cream was easy to remove by Extrudability reach to 93%.

3.1. Irritancy test

There was no sign of redness and itching and safe for topical application.

Table 2 Physical properties of HaE cream.

Color	White yellowish
Odor	Odorless
Appearance	Semisolid
Consistency	Smooth
Felling	Cold
Loss on drying (%)	15.4
pH	7
Spread ability (sec)	38 gcm/sec
Diffusion study (cm)	0.3
Phase separation	at medium and high speed
Viscosity (cps)	25240
Density (g/cm ³)	0.89

Thermal Stability	Stable
Stability	Stable at 37°, 25° and 2°c
Solubility	Insoluble in (alcohol, ether, DMSO, acetone) Soluble in xylole
Irritancy	Non-irritant
Extrudability	93%

3.2. Free radical scavenging activity

The results of DPPH scavenging activity of HaE cream are shown in the figure 1. The radical scavenging activities were estimated by comparing the percentage of inhibition of DPPH radicals by the cream. The present results showed that HaE cream has in vitro antioxidant activity as indicated by dose dependent inhibition of DPPH radicals in range from 57 to 70 %.

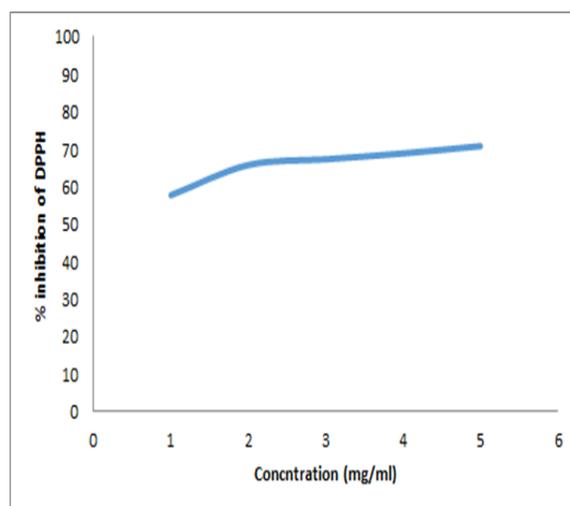


Figure 1 Radical scavenging activity of HaE cream

3.3. Nitric oxide (NO) scavenging activity

The results of NO scavenging activity of HaE cream are shown in the figure 2. The present results showed that HaE cream has in vitro NO scavenging activity in range from 25 to 49 %.

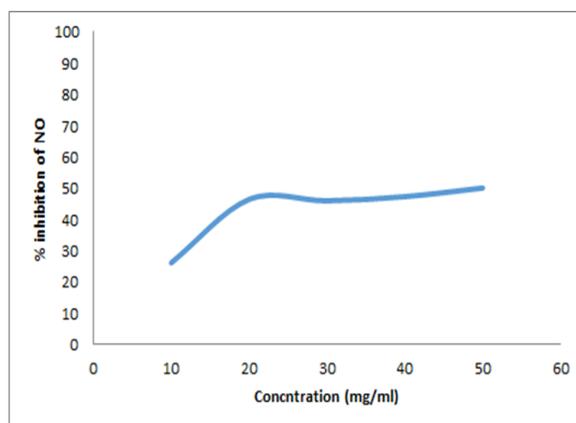


Figure 2 NO scavenging activity of HaE cream

4. Discussion

For the therapeutic strategies of skin-aging and age-related skin diseases, it is important to find an antioxidant compound that is able to be applied to the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance without affecting body structure or functions. During the course of evolution, many invertebrates have been established as a selective advantage by endogenous production of protective chemicals. Sea cucumbers are rich in several bioactive compounds, including saponin, chondroitin sulfate, collagen, amino acids, and phenols. These bioactive compounds have diverse functional roles as a secondary metabolite and these properties can be applied to the developments of novel cosmeceuticals. Therefore, the present study developed a topical skincare cream (w/o) loaded with the sea cucumber, *Holothuria arenicola* (HaE) extract.

Oxidative stress has been suggested to play a role in ultraviolet (UV) damage mediated melanogenesis and reduction on skin elasticity [22]. The application of active antioxidant substances in cosmetic formulations stems from their ability to protect the skin against oxidative damage by UV radiation and aging. Most skincare formulations claiming anti-aging effects are based on exogenous antioxidants such as vitamins, polyphenols, and flavonoids that cannot be synthesized by our body. A direct idea about the antioxidant competence of a compound can be obtained by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays. DPPH is a synthetic-free radical which can be effectively scavenged by antioxidants [23]. It has been widely used for rapid evaluation of the antioxidant activity of cream formulations [24]. In the present study, the ability of formulated HaE cream to scavenging DPPH radicals was determined by the decrease in its absorbance at 517 nm. The overproduction and metabolites of nitric oxide (NO) have been implicated in the development of pathologies although it is extremely important to defend the body [25]. Therefore, NO production blocking agents might be beneficial for the treatment of the inflammatory response. The antioxidant activity of the formulated HaE cream may be due to its high content of antioxidant phenolic compound, chlorogenic acid. Phenolic compounds represent one of the most important antioxidants which play an important role as free radical scavengers, reducing agents, and quenchers.

The pH is a significant parameter as far as the effectiveness of the cream is concerned. The skin is naturally acidic in nature as it typically ranges from 4.5 to 6.0 [26] and this acidity plays an important role in the skin's defense mechanisms and overall skin health. The present study showed that formulated *H. arenicola* cream was found homogenous, easily washable, and had neutral pH which was compatible with normal skin physiology.

Spreadability consists in the expansion of a semi-solid formulation on a surface after a certain time, and its determination is important because topical products should be easy to apply on the skin surface [27]. The results obtained showed that the prepared cream having a good appearance, smooth texture, and easily spreadable property. The low value of the spreadability coefficient of the cream was sufficient suggesting easy spreading. The lower value of spreadability indicates the lesser work required to spread the cream over the skin, which means the formulation was easily spreadable by applying a small amount of shear. Viscosity is a useful process indicator of emulsion quality, as it is highly sensitive to changes in the emulsion due to variations in process and formulation parameters. The viscosity of formulated HaE cream was found at 25240 cps which indicates that the cream is easily spreadable by small amounts of shear. It has been reported that increase the viscosity of emulsion formulations is an important approach that is used to ensure a more controlled application of products, and the production of a uniform, thick, and effective sunscreen film on the skin [28]. In addition, no phase separation was observed for the formulated HaE cream stored at different conditions for 28 days. This indicated stable formulation, considering phase separation as a parameter of stability. Depending on the conditions, the cream may be more stable at a lower temperature due to increased phase viscosity.

In conclusion, the formulated HaE cream was successfully developed that met the relevant pharmaceutical characteristics form. The developed HaE cream could be added in topical formulations in order to protect skin against damage caused by free radicals due to its antioxidant activity. The developed HaE cream is a potential candidate for conducting further clinical studies.

4.1.1. The Conflict of Interest

- Ayman Saber Mohamed disclose that there are no conflict of interest
- Sohair Ramadan Fahmy disclose that there are no conflict of interest
- Abdelaziz A. A. Elsayed disclose that there are no conflict of interest

Compliance with ethical standards

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Disclosure of conflict of interest

The Conflict of Interest

All authors disclose that they have no conflict of interest

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