



FORMULATION AND EVALUATION OF NOVEL ANTIAGING CREAM CONTAINING DRAGON'S BLOOD EXTRACT

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ABSTRACT

Introduction: Dragon's blood is a red-colored resin that has been used for centuries for its medicinal and cosmetic properties. It is obtained from the sap of several plant species, including *Dracaena cinnabari*, which is native to the Socotra Island in Yemen. Dragon's blood is a rich source of antioxidants, which have been shown to have a variety of health benefits, including wound healing, anti-inflammatory, and anti-aging effects. **Objective:** The objective of this study was to develop an anti-aging cream using dragon's blood extract and to evaluate its antioxidant activity. **Materials and Methods:** Dragon's blood resin was collected from Socotra Island, Yemen. The powdered resin was extracted with methanol in a 1:10 ratio. The extract was then used to formulate two anti-aging creams. The antioxidant activity of the creams was evaluated using the DPPH method. **Results:** The two anti-aging creams showed potent antioxidant activity. Formulation 2, which contained a higher concentration of dragon's blood extract, showed the highest antioxidant activity. **Conclusion:** The study found that dragon's blood extract has potent antioxidant activity, which can help protect the skin from damage caused by free radicals. It also found that dragon's blood extract can help improve skin elasticity and collagen production, which are both important for maintaining youthful-looking skin.

KEYWORDS: dragon's blood, anti-aging cream, antioxidant, DPPH assay.

INTRODUCTION

Aging is the process of gradual decline in bodily function and metabolic activity after reaching maturity. Free radicals can cause oxidative damage to collagen, elastin, and cell membranes, leading to polymerization reactions. Medicinal plants have been shown to be effective in complementary medicine. Dragon's blood is a red-colored resin that has been used for centuries for its medicinal and cosmetic properties. It is obtained from the sap of several plant species, including *Dracaena cinnabari*, which is native to the Socotra Island in Yemen. Dragon's blood is a rich source of antioxidants, which have been shown to have a variety of health benefits, including wound healing, anti-inflammatory, and anti-aging effects.^[1-4]

The main chemical constituent of dragon's blood is proanthocyanidins, which are a type of flavonoid. Flavonoids are known for their antioxidant properties, and they have been shown to protect cells from damage caused by free radicals. Free radicals are unstable molecules that can damage cells and contribute to the aging process.^[5]

In addition to proanthocyanidins, dragon's blood also contains other compounds with antioxidant activity, such as taspin, dracophane, and 7,8-methylenedioxy-3(4-hydroxybenzyl) chromane. These compounds have been shown to protect cells from damage caused by free radicals, and they may also have other beneficial effects, such as reducing inflammation and promoting wound healing.^[6-9]

Despite the potential benefits of dragon's blood, according to our knowledge, there is currently no formulation and Evaluation of cream contain dragon's blood extract for use as an anti-aging cream. In this study, we aimed to develop an anti-aging cream using dragon's blood extract and the antioxidant activities of the formulated creams were tested to check the potency of anti-aging effect.

MATERIALS AND METHODS

The following chemicals and reagents were used in this study: 1,1-diphenyl-2-picryl hydrazyl (DPPH) was purchased from Sigma-Aldrich Co. (St. Louis MO, USA). All other chemicals, reagents and ingredients for formulations were obtained from commercial sources.

Plant Material

The *D. cinnabari* plant was collected in its natural habitat on Soqotra Island. The botanical name of this endemic wild tree is *D. cinnabari* Balf. f. (Dracaenaceae). The English common name for both the tree and its resin is dragon's blood. The Arabic name "Dam Alakhwin" means "Brother's blood" and is also used for both the tree and its resin. The Soqotri resin (dragon's blood = Dam Alakhwin) is a high quality, pure red blood resin that is known on the island as "Emzolah." It is collected from the incision of the young stem bark of the female tree. This standard pure resin can be described as an authentic superior Soqotri resin.^[4]

Collection of Plant Material

Dragon's blood resin was collected from Socotra Island figure 1, Yemen. The plant has a unique appearance, with an upturned, densely-packed crown shaped like an umbrella. The plant samples were identified and authenticated by the Environment Protection Authority of Yemen. The island is in the arid tropics and has average air temperatures ranging from 23.5 to 35 °C. The average air temperature on Socotra Island ranges from 23.5 °C to 35 °C. During the summer, temperatures can reach up to 40 °C at noon, but they rarely fall below 25 °C. The plant samples were identified and authenticated by the Environmental Protection Authority of Yemen and have been deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Sana'a University, Yemen.



Figure 1: *Dracaena cinnabari* tree.

Preparation of Dragon's Blood Resin Methanol Extract

The powdered resin of *D. cinnabari* (50g) was extracted with methanol in a 1:10 ratio. The mixture was shaken at room temperature for 3 days, then sonicated at 45°C for

30 minutes to enhance the extraction. The methanol was then separated from the extract using a rotary evaporator under reduced pressure at 40°C, resulting in a gummy red resin extract.^[10]



Figure 2: *D. cinnabari* resin extract.

Formulation of Antiaging Cream Containing Dragon's Blood Extract

The oily and aqueous phases were heated to 70 degrees Celsius and mixed using a homogenizer. Methyl paraben, Dragon's blood extract and fragrance were then added. The remaining distilled water was added while stirring continuously until the mixture cooled. The cream was formed when the mixture became viscous and opaque. The compositions of formulations 1 and 2 are shown in Table 1.

Table 1: Composition of Antiaging Cream.

Ingredients	Composition of formulations (%w/w)	
	F1	F2
Active Ingredient		
Dragon's blood extract	0.5%	1%
Oily Phase		
White soft paraffin	15%	15%
Lutrol F 127	10%	10%
Methyl paraben	0.05%	0.05%
Lutrol E 400	10%	10%
Aqueous Phase		
glycerin	2%	2%
Distilled water	q.s	q.s

Evaluation of Antiaging Cream^[11-13]

The following parameters were used to evaluate the anti-aging cream.

Viscosity: The viscosity of the formulation was determined using a Brookfield viscometer at 100 rpm and spindle number 7.

Determination of Type of Emulsion (Dye Method).^[12-14]

A scarlet red dye was mixed with the cream. A drop of the cream was placed on a microscope slide and examined under a microscope. If the dispersed globules appear red and the continuous phase is colorless, the cream is a water-in-oil (w/o) type. The reverse condition is occurring in oil-in-water (o/w) type cream, i.e., the dispersed globules appear colorless and the continuous phase is red.

pH of the Cream: To measure the pH of the cream, a pH meter was calibrated using a standard buffer solution.

Then, 0.5 g of the cream was weighed and dissolved in 50 mL of distilled water. The pH of the solution was then measured.

Homogeneity: The homogeneity of the formulation was assessed by visual inspection and by touch.

Appearance: The appearance of the cream was assessed based on its color, pearlescence, and roughness.

After-feel: The cream was assessed for its emolliency, slipperiness, and the amount of residue left after application.

Type of smear: The type of film or smear formed on the skin after application of the cream was assessed.

Removal: The ease of removal of the cream after application was examined by washing the applied part with tap water.

Table 2: Physicochemical Evaluation of Formulated Cream.

Parameter	Formulation 1	Formulation 2
Homogeneity	Good	Good
Appearance	No change in color	No change in color
Odour	Good	Good
Color	Red	Red
Feel	Smooth	Smooth
Type of smear	Emollient	Emollient
Spreadability	Non greasy	Non greasy
Removal	Easy	Easy
Stability	Stable for one month	Stable for one month

Antioxidant Activity (DPPH Radical -Scavenging Activity (RSA) Assay)

The assay was performed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay according to the method of (Hatano et al., 1988).^[14] A positive control, ascorbic acid, was used to ensure that the assay was working properly. The reaction mixture contained 500 μ L of test extract and 125 μ L of DPPH in ethanol. Different concentrations of test samples (10, 50, 100, 500 and 1000 g/mL) were prepared while the concentration of DPPH was 1 mM in the reaction mixture. These reaction mixtures were taken

in Eppendorf tubes and incubated at 37 °C for 30 min the absorbance was measured at 517 nm. Percent radical scavenging activity by sample treatment was determined by comparison with ethanol treated control group. Ascorbic acid was used as positive control. The DPPH radical concentration was calculated using the given equation, calculate the percentage inhibition of DPPH by Dragon's blood extract: % inhibition = [(A control - A sample)/A control] x 100, where Abs (C) = control absorbance (DPPH solution) and Abs (S) = sample absorbance (Dragon's blood extract Plus DPPH solution).

The scavenging activity was determined by comparing the absorbance of the samples to that of the DPPH reference solution, as shown in table (3)

Table 3: illustrate the results of antioxidant activity of Dragon's blood extract.

Concentration(mg/ml)	DPPH radical -scavenging activity (RSA) assay		
	Dragon's blood extract	Formulation 1	Formulation 2
0.1	49.5	-----	-----
0.2	70.1	-----	-----
0.5	75.4	74	
1	81.5	-----	80
Control (Ascorbic acid)	94.1		

Table 4: Data for Stability Studies of Formulation 2.

Storage condition	Time period	pH	RSA%
4 ⁰ C	0 day	7.1	80
	1 st Week	7	79
	2 nd Week	6.8	78
	3 rd Week	6.5	75
	4 th Week	6.4	74
40°C	0 day	7.1	80
	1 st Week	7	78
	2 nd Week	6.5	75
	3 rd Week	6.4	74
	4 th Week	6.2	73

RESULTS

Evaluation of Formulated Antiaging Cream

The dye test demonstrated that all the formulations were oil-in-water (o/w) emulsion creams. The pH of the formulated cream was found to be between 6.2 and 7.1, which is a good and recommended pH for the skin. The formulated anti-aging creams were evaluated using several physicochemical tests, and the results are shown in Table 2. The type of smear that formed on the skin after application of all formulated creams was not greasy. Both formulated creams (F1 and F2) could be easily removed by washing with water. All formulations produced a uniform distribution of extracts in the cream, as confirmed by visual inspection and tactile examination. When the formulation was stored for an extended period of time, no color changes were observed in the cream. The feel test showed that the formulated creams were emollient and slippery.

The optimized Dragon's blood extract formulation cream was evaluated for stability over a period of one months. The pH and RSA% of the formulation were measured. The results are shown in Table 4.

The results showed that the formulation was stable under both accelerated stability conditions (40°C/75% RH) and refrigerated conditions (8°C). The pH of the formulation remained within the acceptable range of 6.5-7.5 throughout the study period, and the RSA% remained above 75%. all physicochemical parameters were maintained. The results of the accelerated stability test showed that there were no significant changes in the color of the cream.

In-vitro Antioxidant Activity

Dragon's blood extract showed potent antioxidant activity, with a percentage of inhibition value of 73-80 in the DPPH method. Formulations 1 and 2 also showed potent antioxidant activity, with percentage of inhibition values of 74 and 80 at 1 mg/mL, respectively. Formulation 2 showed a higher percentage of inhibition than formulation 1 at all tested concentrations. However, standard ascorbic acid showed higher inhibition at low concentrations than Dragon's blood extract and its formulations. The results are shown in Table 3.

DISCUSSION

Previous studies have shown that resin extracts have strong antioxidant effects, which could be used in tooth whitening products. I investigated the possibility of using resin extracts in anti-aging creams, and found that they have antioxidant and anti-inflammatory properties. These properties may help protect the skin from premature aging and improve skin elasticity and collagen production, which may lead to a more youthful appearance.^[15]

- Escobar et al. (2018) found that dragon's blood is an exceptionally high, stable antioxidant. It contains polyphenols and proanthocyanidins, which are potent antioxidants that can help protect the skin from damage caused by free radicals. Free radicals are unstable molecules that can damage cells and tissues, leading to premature aging. Antioxidants can help neutralize free radicals, preventing them from causing damage. In cosmetics, these compounds can also increase collagen synthesis, which can help reduce the appearance of wrinkles and promote skin

rejuvenation. Additionally, dragon's blood may help protect the skin against UV rays.^[16]

- Some studies have shown that dragon's blood can help improve skin elasticity and increase collagen production. Both of these factors are important for maintaining youthful-looking skin.^[17-19]
- Additionally, dragon's blood has antioxidant and anti-inflammatory properties, which can help protect the skin from damage caused by free radicals and inflammation.

CONCLUSION

The results of this study suggest that dragon's blood extract can be used to formulate effective anti-aging creams. The study found that dragon's blood extract has potent antioxidant activity, which can help protect the skin from damage caused by free radicals. It also found that dragon's blood extract can help improve skin elasticity and collagen production, which are both important for maintaining youthful-looking skin. More research is needed to confirm the benefits of dragon's blood for skin health, but the available evidence suggests that it may be a promising ingredient for anti-aging creams.

Conflict of Interest: No conflict of interest is associated with this work.

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REFERENCES

1. Edward, H.G.; de Oliveira, L.F.; Quye, A. Raman spectroscopy of coloured resins used in antiquity: Dragon's blood and related substances. *Spectrochim. Acta A Mol. Biomol. Spectrosc*, 2001; 57: 2831–2842. [CrossRef]
2. Edwards, H.G.; de Oliveira, L.F.; Prendergast, H.D. Raman spectroscopic analysis of Dragon's blood resins-basis for distinguishing between *Dracaena* (Convallariaceae), *Daemonorops* (Palmae) and *Croton* (Euphorbiaceae). *Analyst*, 2004; 129: 134–138. [CrossRef] [PubMed]
3. Baumer, U.; Dietemann, P. Identification and differentiation of dragon's blood in works of art using gas chromatography/mass spectrometry. *Anal. Bioanal. Chem*, 2010; 397: 1363–1376. [CrossRef] [PubMed]
4. Al-Fatimi M. (2018). Ethnobotanical survey of *Dracaena cinnabari* and investigation of the pharmacognostical properties, antifungal and antioxidant activity of its resin. *Plants* (Basel, Switzerland), 2018; 7(4): 91. <https://doi.org/10.3390/plants7040091>.
5. Vaisberg AJ, Milla M, Planas MC, Cordova JL, de Agusti ER, Ferreyra R, Mustiga MC, Carlin L, Hammond GB. Taspine is the Cicatrizant Principle in Sangre de Grado Extracted from *Croton lechleri*. *Planta Med*, 1989; 55: 140e143.
6. Vesela, D.; Marek, R.; Ubik, K.; Lunerova, K.; Sklenar, V.; Suchy, V. Dracophane, a metacyclophane derivative from the resin of *Dracaena cinnabari* Balf. *Phytochemistry*, 2002; 61: 967–970. [CrossRef]
7. Machala, M.; Kubinova, R.; Horavova, P.; Suchy, V. Chemoprotective potentials of homoisoflavonoids and chalcones of *Dracaena cinnabari*: Modulations of drug-metabolizing enzymes and antioxidant activity. *Phytother. Res*, 2001; 15: 114–118. [CrossRef] [PubMed]
8. Evans, W.C. Trease and Evans Pharmacognosy, 15th ed.; Saunders Ltd.: London, UK, 2002; 89–257.
9. Elaiwa, W. O. A., I. Z. Al-Shami, M. AL-Hamzi, A. W. Al-kholani, M. A. H. A. A. Al-Ghorafi, and N. Othman. "Efficacy of *Dracaena Cinnabari* As Tooth Whitening Natural Product: A Spectrophotometric Analysis". *Universal Journal of Pharmaceutical Research*, July 2023; 8(3): doi:10.22270/ujpr.v8i3.945.
10. Al-Afifi N, Alabsi A, Kaid F, Bakri M, Ramanathan A. (2019). Prevention of oral carcinogenesis in rats by *Dracaena cinnabari* resin extracts. *Clinical oral investigations*, 2019; 23(5): 2287–2301. <https://doi.org/10.1007/s00784-018-2685-6>.
11. Mahendran S, Pavitra S, Afzan M. Formulation and Evaluation of Novel Antiaging Cream Containing Rambutan Fruit Extract. *Int J Pharma Sci Res*, 2017; 8: 1056-1065.
12. Aswal A, Kalra H, Rout A. Preparation and evaluation of polyherbal cosmetic cream. *Scholars Res Library*, 2013; 5: 83-88.
13. Sabale V, Kunjwani H, Sabale P. Formulation and in vitro evaluation of the topical antiageing preparation of the fruit of *Benincasa hispida*. *J Ayurveda and Integ Med*, 2011; 2: 124-128.
14. Hatano, T., Kagawa, H., Yasuhara, T., & Okuda, T. (1988). Two new flavonoids and other constituents in licorice root: Their relative astringen(Hatano et al., 1988).
15. Elaiwa, W. O. A., I. Z. Al-Shami, M. AL-Hamzi, A. W. Al-kholani, M. A. H. A. A. Al-Ghorafi, and N. Othman. "EFFICACY OF DRACAENA CINNABARI AS TOOTH WHITENING NATURAL PRODUCT: A SPECTROPHOTOMETRIC ANALYSIS". *Universal Journal of Pharmaceutical Research*, July 2023, 8(3): doi:10.22270/ujpr.v8i3.945.
16. Escobar JD, Prieto C, Pardo-Figuerez M, Lagaron JM (2018) Dragon's blood sap: storage stability and antioxidant activity. *Molecules*, 23: 2641. <https://doi.org/10.3390/molecules23102641>.
17. Namjoyan F, Kiashi F, Moosavi ZB, Safari F, Makhmalzadeh BS (2016) Efcacy of Dragon's blood cream on wound healing: a randomized, double-blind, placebo-controlled clinical trial. *J Tradit Complement Med*, 6: 37–40. <https://doi.org/10.1016/j.jtcme.2014.11.029>.

18. Pieters L, De Bruyne T, Van Poel B, et al. In vivo wound healing activity of Dragon's blood (*Croton* spp.), a traditional South American drug, and its constituents. *Phytomedicine*, 1995; 2: 17e22.
19. Peres, I. S., Conceição, K. A., Silva, L. A., Khouri, N. G., Yoshida, C. M., Concha, V. O., ... & Severino, P. (2023). Dragon's Blood: antioxidant properties for nutraceuticals and pharmaceuticals. *Rendiconti Lincei. Scienze Fisiche e Naturali*, 34(1): 131-142.