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ORIGINAL RESEARCH

Preparation of *Centella asiatica* (L.) and *Hypericum perforatum* (St. John's Wort) Plant Extracts and Development of Anti-Aging Herbal Cream Formulations

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Abstract

Objective: The study aims to use two different plant extracts, St. John's Wort (*Hypericum perforatum*) and *Centella asiatica* (L.), in the cream formulation and determine the new formulation's anti-aging effect.

Materials-Methods: The plants used in the study were obtained commercially, and plant extracts were obtained using the classical extraction techniques in the literature. The active ingredients in the obtained extracts were determined by the HPLC method. Physical, protective efficacy, microbial analysis, and anti-aging tests were carried out for cream formulations obtained with extracts.

Results: Plant extraction studies were carried out in the study. *Centella asiatica* (L.) was extracted with a 20.8% yield at 20 mL of methanol and 60°C. As a result of HPLC analysis, it was determined that there were 1740 mg/kg asiatic acid and 4380 mg/kg madecasic acid in the extract. In the extraction studies performed on *Hypericum perforatum*, the expected active ingredient, hypericin, was not found. For this reason, a commercial extract containing 2.5% hyperforin was obtained, and the studies were continued on these extracts, and final cream formulations were obtained.

Conclusion: pH, viscosity, density, protective effectiveness tests, and microbial analysis tests of the final formulations were performed. In addition, in vitro, anti-aging studies have been carried out in accredited laboratories. In-vitro anti-aging tests determined that the level of collagen 1A increased more in the formulation where both extracts were used together.

Keywords: *Hypericum perforatum*, *Centella asiatica* (L.), *In Vitro*, Anti-aging Herbal Cream.

INTRODUCTION

Hypericum perforatum and *Centella asiatica* (L.) have rich contents in terms of polyphenols, flavonoids, and terpenes in terms of active ingredients. This feature emphasizes the power of the antioxidant property of the content. In addition, both ingredients will inevitably have a substantial effect thanks to their anti-inflammatory effect, antioxidant effect, and some triggered reactions in the metabolic pathway. For this reason, after the extracts of both plants were prepared, the types and amounts of active ingredients were determined, and cream formulation studies were carried out. Accredited laboratories have tested appropriate formulas for pH, viscosity, density, protective activity, and microbial analysis. Afterward, in-vitro anti-aging tests were carried out.

For this reason, after the extracts of both plants were prepared, the types and amounts of active ingredients were determined, and cream formulation studies were carried out. For suitable formulas, pH, viscosity, density, protective activity tests, and microbial analyses were performed. In-vitro anti-aging tests were carried out. According to the results, it has been demonstrated that its anti-aging feature is vital. *C. asiatica* (L.) and *H. perforatum* are highly beneficial herbs in traditional medicine and cosmeceuticals.

C. asiatica (L.) contains flavonoids, phenolic acids, steroids, amino acids, vitamins, and essential oils. Madecassoside, asiaticoside, and their sapogenin triterpene acids (madecassic and asiatic acid) are the components used as biomarkers to evaluate the quality of the *C. asiatica* (L.) plant and responsible for most of its pharmacological activity (Figure 1).[1,2] These compounds are considered active ingredients in the pharmaceutical industry. In addition, *C. asiatica* (L.) is a rich source of antioxidants as it contains many phenolic compounds such as quercetin, catechin, luteolin, rutin, kaempferol, myricetin, naringin, and naringenin.[3]

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C. asiatica (L.), known as Cica, has become popular in cosmetics in skin care products, antiaging medications, moisturizers, anti-inflammatory drugs, and for treating scars, scars, and atopic dermatitis.[4]

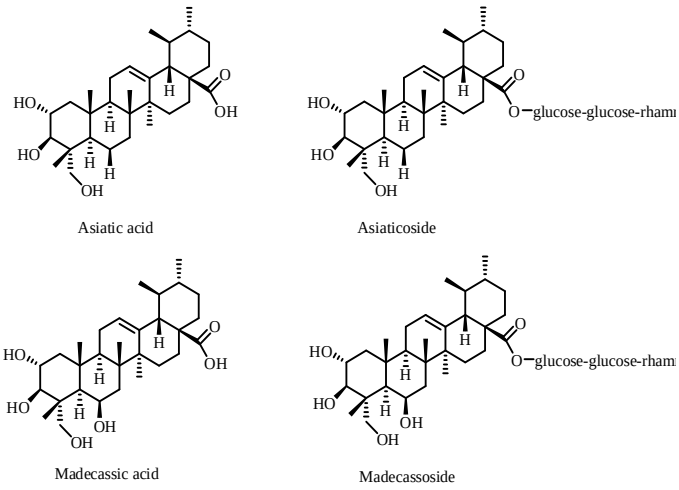

Figure 1. Important compounds for *Centella asiatica* (L.) plant.

Clinical studies have also shown that these effects are due to the triterpenoids in the CA content.[5] Especially *C. asiatica* (L.) has been used in treating skin disorders, and research has focused on this. In scar tissue, asiaticoside, and madecassoside are thought to increase glycosaminoglycan synthesis, hydroxyproline, and collagen content (Figure 1). Besides wound healing in cosmeceuticals, they are also used for anti-wrinkle and anti-cellulite effects as they improve fibronectin production in human skin fibroblasts and increase collagen synthesis[1,6]. Madecassoside, a triterpene in *C. asiatica* (L.), has also been reported to inhibit UV-induced hyperpigmentation in human skin cells.[2] Apart from this, it has been shown to exhibit an antiaging effect[7], memory-enhancing effect against Alzheimer's[8], anti-hypertensive effect[9], diabetes, anti-cancer[10], cardioprotective effect[11], antibacterial effect[12], anti-inflammatory effect[13], antioxidant[14] and anti-ulcer properties.[1,15]

C. asiatica (L.) has been suggested in traditional medicine for treating various skin conditions such as leprosy, lupus, varicose ulcers, eczema, psoriasis, diarrhea, and fever.[16] It has also been used in traditional medicine to treat neurological diseases such as dementia, Alzheimer's, anxiety, and depression.[17]

H. perforatum, also known as St John's Wort, contains a variety of bioactive substances such as hypericin, chlorogenic, caffeic acids, flavonoids, quercetin, biapigenin, procyanidin, and hyperforin[18] (Figure 2.). Although hypericin and hyperforin, the main components of *H. perforatum*, are the focus of attention due to their antidepressant effects, they also have an essential place in anti-aging studies.[19-22] However, Hypericins are unstable and are easily converted to their different derivatives.[23] It has been shown to strengthen the analgesia caused by morphine with the hypericin it contains.[24] Furthermore, it has been shown in the literature to significantly affect wound healing, acne treatment, and skin disorders such as eczema and psoriasis.[25] Moreover, very positive feedback has been received for the extract of this plant, which has been used in cosmetic products recently. Apart from these, it has been shown that *H. perforatum* extracts can exhibit multiple bioactivities, including anti-inflammatory[26], antioxidant[27], antibacterial[28], antifungal[18], and anti-cancer.[29] It is also effective in digestive problems.[30]

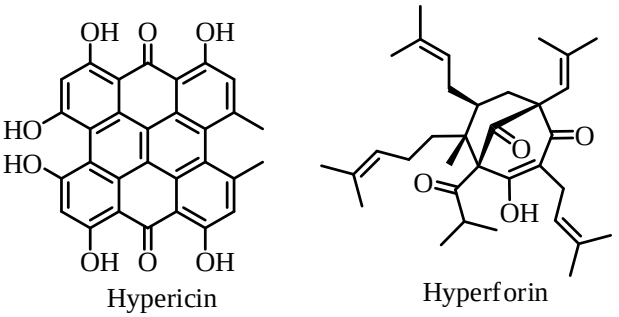

Figure 2. Two important compounds in *Hypericum perforatum*; Hypericin and Hyperforin.

Skin aging is a biological process and is inevitable. Collagen fibers give the skin tensile strength; elastin fibers contribute to its flexibility and durability. The decrease in protein synthesis, which affects Type I and III collagen, is also very effective in this process. Furthermore, skin aging is how these properties change depending on intrinsic and extrinsic aging factors[31]. Skin aging increases due to sun, toxins, air pollution, inflammation, gravity effect, muscle loss, decrease in collagen content, DNA damage, telomere loss, and oxidative stress.[32] However, some applications can delay this process based on inhibiting the function of degrading enzymes such as collagenase, hyaluronidase, and elastase. Specially synthesized molecules and some plant extracts are used for this purpose. Active substances, frequently used in cosmetic products and food supplements, also take their place in medical applications. The most accessible and most practical of anti-aging applications is anti-aging creams.

MATERIALS AND METHODS

Sample preparation

Centella asiatica and *Hypericum perforatum* plants used in the study were supplied as dry from Aksuvital Natural Products Food Industry and Trade Inc.

Solvents such as hexane (96%), ethanol (99.9%), acetone (99.5%), and methanol (99.8%) were obtained from ISOLAB and used without any processing.

The extraction method of *Centella asiatica* (L.)

1 g of plant was mixed vigorously in 40 ml of methanol at 60 ℃ for 3 hours under reflux in a magnetic stirrer. After the mixing process was completed, filtration was carried out with the help of filter paper without cooling the mixture. After the solvent in the filtrate was removed with the aid of an evaporator, the remaining solid was weighed.[33] The solid part was stored at +4 ℃ for later use.

Extraction method of Hypericum perforatum

200 ml of methanol was added to 5 g of plant and mixed vigorously in a magnetic stirrer under reflux for 6 hours at 60 ℃. After the mixing process was completed, filtration was carried out with the help of filter paper without cooling the mixture. After the solvent in the filtrate was removed with the aid of an evaporator, the remaining solid was weighed.[34] The solid part was stored at +4℃ for later use.

Instruments for HPLC

For HPLC/DAD analysis, the Shimadzu LC 20AT HPLC system is equipped with the SPD-M20A photodiode array detector (DAD). The samples were separated on a C18 250*4.6 mm column.

HPLC method for *Centella asiatica* (L.)

C18 250*4.6 mm column, 1.0 ml/min flow rate, 30℃ column temperature, with 210 nm UV detector, acetonitrile/pure water (contains 0.1% 85% o-phosphoric acid) (v/v= 1/1) HPLC analysis was carried out in the) solvent mixture and for 20 min18.

HPLC Method for *Hypericum perforatum*

C18 250*4.6 mm column, 1.0 ml/min flow rate, 30ºC column temperature, 210 nm UV detector, acetonitrile/pure water (0.1% formic acid contains (v/v=1/1) in the solvent mixture and HPLC analysis was performed for 20 min18.

Cream formulation studies

All excipients and chemicals used in cream production were obtained from Türkiye, Sigma-Aldrich® (Glycerine) and Basf companies (Sodium polyacrylate, Dicaprylyl carbonate, Polyglyceryl-3 caprate Coco caprylate, Sodium Stearoyl Glutamate, Sorbitan Caprylate, Propanediol, Benzoic acid, fragrance agent). *Hypericum perforatum* extract was obtained from Aksu Vital Company.

Anti aging herbal cream

After the extracts and ratios were determined for cream production, cream formulations were created (Table 1 and 2). Cream containing *Hypericum perforatum* and *Centella asiatica* (L.) extract was prepared according to the formulation given in Table 1.

Table 1. Formulation for cream production.

	Ingredient	Amount (%) Experiment I	Experiment II	Experiment III
Phase I	*Hypericum perforatum* Extract	-	0.07-0.13	0.07-0.13
	Centella asiatica Extract	0.07-0.13	-	0.07-0.13
	Glycerine	2.5 –3.5	2.5 –3.5	2.5 –3.5
	Sodium Polyacrylate, Dicaprylyl Carbonate, Polyglyceryl-3 Caprate	0.625 – 2.2	0.625 – 2.2	0.625 – 2.2
	Fragrance Agent	1.25 – 2.5	1.25 – 2.5	1.25 – 2.5
	Water	70-80	70-80	70-80
Phase II	Coco Caprylate	5 – 7.5	5 – 7.5	5 – 7.5
	Sodium Stearoyl Glutamate	0.3 – 0.7	0.3 – 0.7	0.3 – 0.7
	Sorbitan Caprylate, Propanediol, Benzoic Acid	0.5-1.3	0.5-1.3	0.5-1.3

The data in Table 1 were prepared for 400 g of plant extract cream. Experiment I is a formulation made with Centella asiatica (L.) extract. Experiment II is a formulation made with *Hypericum perforatum* extract. Experiment III is a formulation with both plant extracts (Table 1). These three experiments were carried out to compare the anti-aging effect.

For each trial (Experiment I, II, and III), the material of each phase was mixed in different containers. After homogenizing each phase mixture, Phase I and II were combined. The phases were mixed until a homogeneous and dense cream consistency. Three separate trials have been formulated for anti-aging studies. Experiment III was determined as the final formulation as it was demonstrated by anti-aging studies that it was more adequate than the others (Experiments I and II).

In Table 2, the plant species used and the major active

ingredients in it, the amounts in the extract are given. These rates in Table 2 are presented by comparing them with previous studies in the literature.[20,31]

Table 2. The plant species used, the ratio of active ingredient in the extract.

Plant Type	Active Substance	Amount in Extract
Centella asiatica (L.)	Asiatic acid	0.17
	Madecasic acid	0.44
Hypericum perforatum	Hyperforin	2.5

In addition to extracts, different natural ingredients such as thickeners, emulsifiers and preservatives are used in cream formulations. In the formulation, glycerin is an effective ingredient both for its moisturizing properties and as a solvent. The mixture of sodium polyacrylate, Dicapryl carbonate, Polyglyceryl-3 caprate components were used as thickening agents. Coco caprylate is used to support glycerine and as a substrate moisturizer. At the same time, it contributes to the dissolution of the extract containing the active substance. Sodium Stearoyl Glutamate was used as an emulsifier to combine the oil and water phase. Ingredients containing Sorbitan Caprylate, Propanediol, Benzoic acid are also included in the formulation for preservative purposes (Table 1).

***In vitro* skin antiaging cell culture studies test material**

Human skin fibroblast cell line HS68 (ATCC CRL-1635) obtained from ATCC was used in all experiments within the scope of the study. Cell culture study was carried out for Anti Aging Herbal Cream (sample 10g) formulated with 0.5-4% *Hypericum perforatum* and *Centella asiatica* (L.) extract.

Anti-aging cell culture studies on the cream produced with *Hypericum perforatum* extract (Experiment I)

Test material information (TM)

Hypericum perforatum Extract Herbal Cream Content Information: Available in Table 1 (*Hypericum Perforatum* Flower/Leaf Extract Active Ingredient Ratio: (St. John's Wort Extract) 0.5-3%).

Negative Control (NC): Ultrapure water

Test Material Application doses (w/v): 25, 50 and 100 µg/ml.

Anti-aging studies on cream produced with *Centella asiatica* extract (Experiment II)

Test material information (TM)

Centella asiatica (L.) extract *Herbal Cream Ingredients Information:* Available in Table 1(*Centella asiatica* (L.) Flower /Leaf/Stem Extract, Substance Ratio: (*Centella asiatica* (L.) Extract) 0.5-3%).

Negative Control (NC): Ultrapure water

Test Material Application doses (w/v): 25, 50 and 100 µg/ml

Anti aging studies on cream produced with both extracts (Experiment III)

Test material information (TM)

Centella asiatica Extract Herbal Cream Ingredients Information: Available in Table 1 (*Centella asiatica* (L.) Flower /Leaf/Stem Extract, *Hypericum perforatum* Flower/Leaf Extract Active Ingredient Ratio: (*Hypericum perforatum* and *Centella asiatica* (L.) Extract) 0.5-4%).

Negative Control (NC): Ultrapure water

Test Material Application doses (w/v): 25, 50 and 100 µg/ml

After the cream, was exposed to certain conditions, the soluble part was taken and anti-aging studies were continued. The test material was dissolved in DMEM medium containing 0.05% DMSO. It was incubated in an oven at 37 ℃ for 24 hours. At the end of the period, it was passed through a 0.22 µm membrane filter and used at test concentrations.

***In vitro* skin antiaging cell culture conditions**

Cells were grown in DMEM (ATCC Cat No: 30-2006) growth medium supplemented with 10% FBS and 2% glutamine and incubated at 37°C in an oven with 5% CO2. A mixture of 0.25% trypsin and 0.03% EDTA was used for trypsinization of cells as recommended by ATCC. Cells were divided into 6-well plates at 2x105 cells per well. The amounts of Collagen αI (Col I) released from the medium from HS68 cells were determined using the Human Collagen αI ELISA Kit after 48 hours of incubation. Before starting the experiment, each well was washed four times with 300 µL of 1x wash buffer. 50 µL of the test material was taken from the experimental and control groups at the determined doses and added to these wells. It was incubated on a shaker at 200 rpm for two hours at room temperature. Each well was washed four times with 300 µL of 1x wash buffer. Then, 100 µL of Human Col I antibody was added to each well. It was incubated for one hour at room temperature with a shaker. Each well was washed four times with 300 µL of 1x wash buffer. 100 µL of Avidin-HRP A (Avidin Peroxidase A) solution was added to each well and incubated for 30 minutes at room temperature with a shaker. Each well was washed five times with 300 µL of 1x wash buffer. 100 µL of Substrate F (high sensitivity TMB) solution was added to each well and incubated for 10 minutes

at room temperature and in the dark. Afterward, the blue color formation was observed depending on the amount of Col I bound to the wells. The reaction was stopped by adding 100 µL of stop solution to each well, and the color changed from blue to yellow. The absorbance values of the samples were read in an Eliza kit reader (Thermo Fisher, Multi Scan FC Microplate Reader) at 450 nm.

RESULTS

Extraction and analysis studies

Hypericum perforatum and *Centella asiatica* (L.) plant were extracted at different temperatures and in a polar solvent such as methanol. The extractions performed and their results are available in the table below (Table 3).

Based on the yields in the table, HPLC analysis of the solid extracts were made and the active ingredient contents and amounts were tried to be determined.

According to the results of HPLC analysis; asiatic acid and madecasic acid were determined as the active substances we expected in the *Centella asiatica* (L.) plant (Table 4).

If the results in Table 3 are to be interpreted; The amount of solid extract obtained from 0.5 grams of *Centella asiatica* (L.) with a yield of 20.8% was 0.104 grams (Table 3, Entry 3). When matched with the HPLC results in Table 4, it is understood that there are 0.18 mg of asiatic acid and 0.45 mg of made cacid acid in 0.5 grams of *Centella asiatica* (L.) plant.

Table 3. *Hypericum perforatum* and *Centella asiatica* (L.) plant extraction results.

Entry	Solvent	Amount of Solvent	Temperature ℃	TimeH	Weight	*Centella asiatica* (L.) Yield, %	*Hypericum perforatum* Yield, %
1	MeOH	20 mL	25	3	0.5 gr	19.5	18.8
2	MeOH	20 mL	40	3	0.5 gr	19.7	19.6
3	MeOH	20 mL	60	3	0.5 gr	20.8	19.3

Table 4. *Centella asiatica* (L.) HPLC Result (Quantitative).

Analysis name	Conclusion	Unit	Method
Asiatic acid	1740	mg/kg	HPLC-DAD
Madecasic acid	4380	mg/kg	HPLC-DAD

Cream production studies

For cream formulations, tests such as biosafety and physical tests must be performed before they are made available. pH, viscosity, density, protective activity tests and microbial analysis tests were performed for the final formulation (Experiment III) (Table 5-8).

Cell culture studies

Cream formulation studies were carried out successfully. Our products have been tested in-vitro. In-vitro tests conducted for 3 different formulations revealed that the anti-aging effect was strengthened in the product mixture.

In this cosmetic formulation; *Staphylococcus aureus, Pseudomonas aureginosa, Escherichia coli, Candida albicans and Aspergillus brasiliesis* correspond to "Criteria A" defined according to ISO 11930:2012.

In summary, Table 6 indicates that the product is protected against the growth of the bacteria described above, that is, against microbial growth.

Table 5. Microbiological Test Results for Anti-aging Herbal Cream.

Parameter	Unit	Microbiological Analysis Result	Standarts Nu	Limit
Total Aerobic Mesophilic Microorganisms*	kob/g – cfu/g	<10	ISO 21149	<100
Staphylococcus aureus *	/1g- ml	Absence/1g-ml	ISO 22718	Absence/1g-ml
*Pseudomonas aureginosa**	/1g- ml	Absence/1g-ml	ISO 22717	Absence/1g-ml
*Escherichia coli**	/1g- ml	Absence/1g-ml	ISO 21150	Absence/1g-ml
*Candida albicans**	/1g- ml	Absence/1g-ml	ISO 18416	Absence/1g-ml
Total Yeast and Moulds*	kob/g – cfu/g	<10	ISO 16212	<100

(*) Parameters column which are covered by the accreditation. According to the results of microbiological analysis, the sample complies with the provisions of the "Guideline on Microbiological Control of Cosmetic Products" (Table 5).

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Table 6. Antimicrobial Protective Efficacy Test Results.

Microorganism	0.Day	7.Day		14.Day		28.Day	
	*N0 (Log)	*N7 (Log)	Log reduction	*N14 (Log)	Log reduction	*N28 (Log)	Log reduction
Staphylococcus aureus NCTC 10788/Lot 030520029	6.04	1.00	5.04	1.00	5.4	1.00	5.04
Pseudomonas aureginosa ATCC 9027/Lot 3270513	6.77	1.00	5.77	1.00	5.77	1.00	5.77
Escherichia coli ATCC 8739/Lot 4835151	6.77	1.00	5.77	1.00	5.77	1.00	5.77
Candida albicans NCPF 3179/Lot 040920028	4.69	1.00	3.69	1.00	3.69	1.00	3.69
Aspergillus brasilinesis NCPF 2275/Lot 020620065	3.30	Not performed		1.00	2.30	1.00	2.30

(*) Number of microorganisms by day.

Table 7. Stability Test Results (First Day Results)

First Day Results	Color	Smell	Appearance	pH	Intensity	Packaging	Microbiological Analysis	Phase Separation
	Light brown	Characteristic	Cream	5.19	1.080	Suitable	Suitable	Not observed

Table 8. Stability Test Results (Following Days)*

Parameters	**1 Week**		**1 Month**		**2 Month**		**3 Month**	
temperature	**25°C**	**45°C**	**25°C**	**45°C**	**25°C**	**45°C**	**25°C**	**45°C**
Color	Light Brown	Light Brown	Light Brown	Light Brown	Light Brown	Light Brown	Light Brown	Light Brown
Odor	Characteristics	Characteristics	Characteristics	Characteristics	Characteristics	Characteristics	Characteristics	Characteristics
Appearance	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream
Ph	5.23	5.18	5.28	5.11	5.19	5.02	5.35	5.03
Density	1.082	1.085	1.084	1.082	1.081	1.081	1.085	1.083
Package	Ok	Ok	Ok	Ok	Ok	Ok	Ok	Ok
Microbiological Analysis	Ok	Ok	Ok	Ok	Ok	Ok	Ok	Ok
Phase Separation	Not Observed	Not Observed	Not Observed	Not Observed	Not Observed	Not Observed	Not Observed	Not Observed

(*) Chemical Result: Density changes at a level that can be ignored. Microbiological Result: The possibility of microbiological growth was not observed. Physical Result: About color change was observed. About odor change was not observed. Color change was observed in the sample from the 1 st month 45°C.

DISCUSSION

The yields obtained in Table 3 are not the active substance yields. The yields here are the methanol solvent and the plant extraction yields obtained at different temperatures. In other words, it is the mixing efficiency with different components as well as active substance. Determination of the amount of active substance was obtained by HPLC analysis and is given in Table 4.

HPLC spectra corresponding to the quantitative results of asiatic acid and madecasic acid in Table 4 are shown in Figure 3. When the plant extracts were compared with the asiatic acid and madecasic acid standards, madecasic acid was found in 6.29 minutes and asiatic acid was obtained in 9.54 minutes.

However, the expected Hypericin could not be detected in *Hypericum perforatum* extract obtained by the methods mentioned in the literature. HPLC data are shown in Figure 4. *Hypericum perforatum* extract is likely to contain some glycoside derivatives other than Hypericin. In this case, *Hypericum perforatum* extract was obtained directly from Aksu Vital. The amount of active substance in the provided extract, hyperforin, HPLC results are clear. There is 2.5% hyperforin in the extract. The product report is provided and attached.

In order to confirm this result, analysis was performed using the HPLC technique in the literature.[18] However, Hypericin could not be detected both in the extract obtained by experimental methods and in the commercially available extract.

Accordingly, there has been a trend towards commercially available extract containing hyperforin active ingredient. Hypericin and hyperforin are two major active ingredients for *Hypericum perforatum* Hyperforin acts as an antimicrobial and anti-inflammatory agent.

The hyperforin molecule is a biologically active molecule and it is supported by the literature that it is an an active component in cream formulations and even in anti-aging studies.[20,33]

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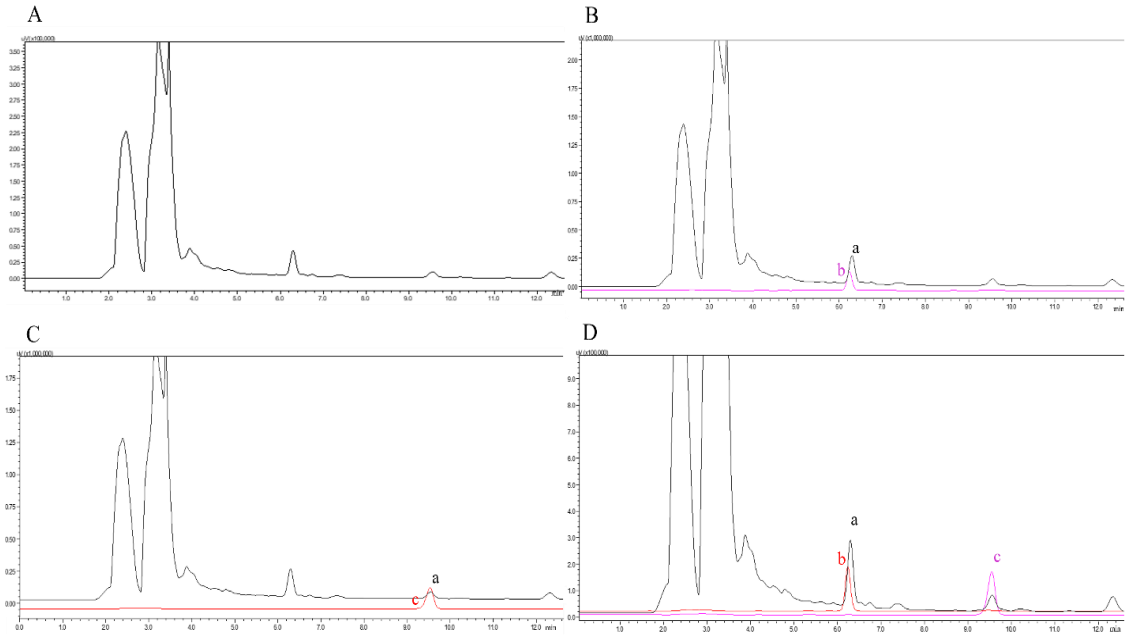


Figure 3. A) *Centella asiatica* (L.) extract Data Spectrum. B) *Centella asiatica* (L.) extract (a-Black) and Madecassic acid Spectrum (b-Pink). C) *Centella asiatica* (L.) extract (a-Black) and asiatic acid Spectrum (c-Red). D) *Centella asiatica* (L.) Extract (a-Black), asiatic acid Spectrum (c-Pink), and madecassic acid Spectrum (b-Red).

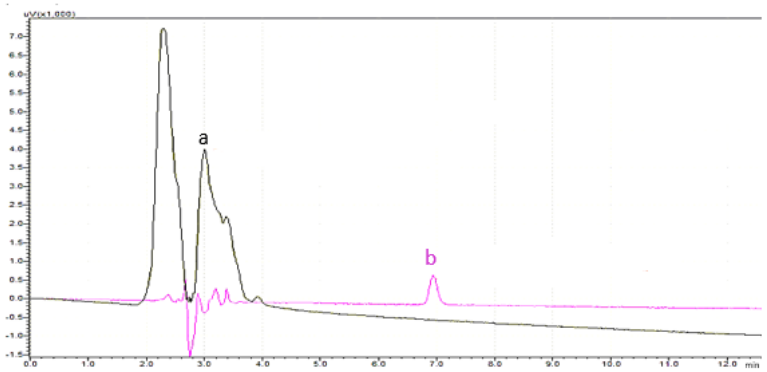


Figure 4. *Hypericum perforatum* extract a-Black data sample and b-pink data hypericin standard.

Extracts were prepared for cream production. For cream production, cream formulations were created after the ratios of *Centella asiatica* (L.) prepared with methanol and commercially available *Hypericum perforatum* extract were determined. In the formulation, besides the extracts, different natural ingredients such as thickeners, emulsifiers and preservatives were used. The contents other than the extract were prepared with the experimental experience and literature support of our group in previous studies, and the extracts were formulated considering the toxicology studies in the literature. The concentrations used in toxicology studies in the literature on *Hypericum perforatum* plant extract were evaluated as the upper limit and formula studies were carried out at values below this limit. In addition, in anti-aging studies, it has been proven that the most effective content is obtained by extraction with methanol.[20,31] Similar literature studies were also carried out for the *Centella asiatica* (L.) plant and the formulation was adapted at values below the working concentrations.[34,35]

Three different cream formulations were prepared for In vitro cell culture study to reveal the anti-aging effect. Experiment I is a formulation made with *Centella asiatica* (L.) extract. Experiment II is a formulation made with *Hypericum perforatum* extract. Experiment III is a formulation created with both plant extracts (Table 1). First of all, after Experiment III was created, after positive results were obtained from the tests made, Experiments I and II were prepared (Table 5-8). As expected, Experiment III with both extracts proved to be a more effective formulation than the others (Experiments I and II).

The most important problem while preparing the

formulations was the homogeneous distribution of the extracts. For this purpose, the extract and/or extract mixtures were primarily dissolved in a mixture of glycerin, coco caprylate, sorbitan caprylate, propanediol, benzoic acid, and a cream formulation was created after a homogeneous mixture was obtained. We have also confirmed with the tests performed that there is no solubility problem as expected.

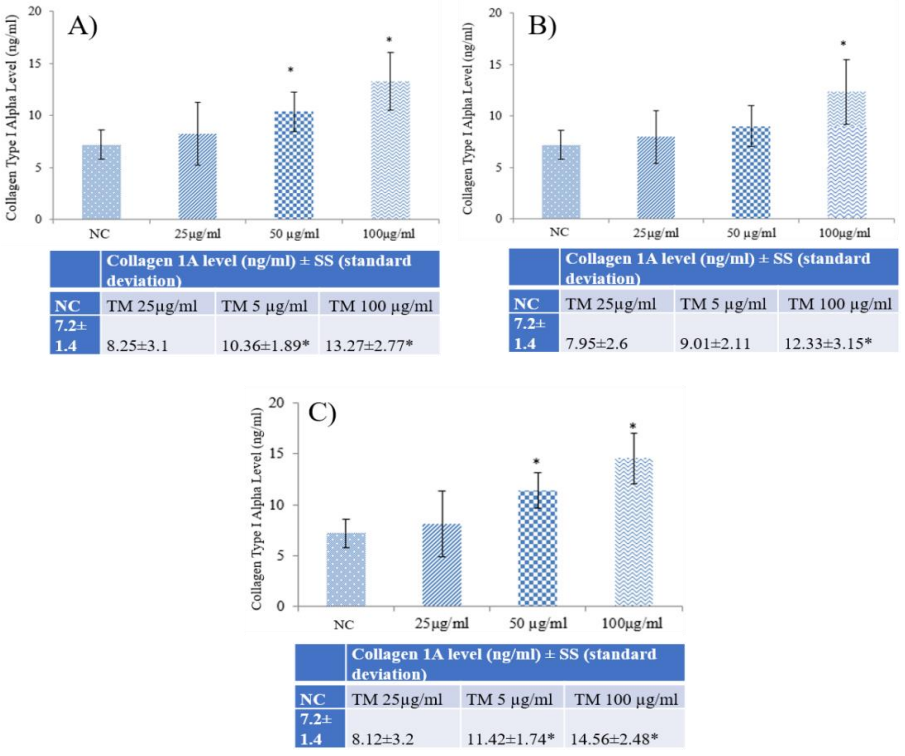
Stability test results are observed in Tables 7 and 8. Microbiological tests must be performed before stability tests. Table 7 presents the analysis of the cream on the first day it reaches the laboratory. In the tests, it has been determined that the cream color is brown, has a characteristic odor, has a pH of 5.19 (this pH is within the desired range), the density is 1.080, the packaging and microbiological analysis are suitable, and most importantly, no phase separation is observed.

In Table 8, when the same measurements were made by exposing to different temperatures (25 °C, 45 °C) in 1 week, 1 month, 2 months and 3 months periods, different results from the first day were not encountered. These results reveal that the product is stable.

The most abundant protein found in all vertebrates is Type I collagen. Collagen, synthesized mainly by fibroblasts, myofibroblasts, osteoblasts and chondrocytes, is a simple and fibrillar scleroprotein found in significant amounts in tendons, cartilage, organic matrix of bones, and cornea of the eye. Anti-aging creams can be used against decreases in the amount of collagen I due to aging. Thus, anti-aging creams can increase the amount of collagen I. Therefore, the amount of collagen I was measured in the test.[36,37]

First of all, anti-aging studies have been carried out on the cream produced only with *Hypericum perforatum* extract. Experiments were repeated as 5 times and the results were given as mean ± standard deviation. Comparisons between groups were made with the Kruskal-Wallis and Mann-Whitney tests. According to these tests, differences with a p value of 0.05 and small were considered statistically significant (*p<0.05).

According to the test results, it was determined that the collagen type I alpha level of *Hypericum perforatum* Extract Herbal Cream at 50 and 100 µg/ml concentrations was statistically increased compared to the control group. According to these results, *Hypericum perforatum* extract Herbal Cream is an effective anti-aging product (Figure 5A).



	Collagen 1A level (ng/ml) ± SS (standard deviation)		
NC	TM 25µg/ml	TM 5 µg/ml	TM 100 µg/ml
7.2± 1.4	8.25±3.1	10.36±1.89*	13.27±2.77*

	Collagen 1A level (ng/ml) ± SS (standard deviation)		
NC	TM 25µg/ml	TM 5 µg/ml	TM 100 µg/ml
7.2± 1.4	7.95±2.6	9.01±2.11	12.33±3.15*

	Collagen 1A level (ng/ml) ± SS (standard deviation)		
NC	TM 25µg/ml	TM 5 µg/ml	TM 100 µg/ml
7.2± 1.4	8.12±3.2	11.42±1.74*	14.56±2.48*

Figure 5. A) Demonstration of dose-dependent changes in the amount of Collagen Type I Alpha from the cream produced only with *Hypericum perforatum* extract in skin fibroblast cells at the end of the 48th hour; B) Dose-dependent change of Collagen Type I Alpha amounts in skin fibroblast cells at the end of 48th hour in cream produced only with *Centella asiatica* (L.) extract; C) The dose-dependent change of Collagen Type I Alpha amounts for the cream prepared by both extractions at the end of the 48th hour in skin fibroblast cells.

Secondly, anti-aging studies were conducted on the cream produced only with *Centella asiatica* (L.) extract. Experiments were repeated as 5 times and the results were given as mean ± standard deviation. Comparisons between groups were made with the Kruskal-Wallis and Mann-Whitney tests. According to these tests, differences with a p value of 0.05 and small were considered statistically significant (*p<0.05).

It was determined that there was a statistical increase in collagen type I alpha level at 100 µg/ml concentration compared to the control group. According to these results, *Centella asiatica* (L.) extract Herbal Cream is an effective anti-aging product (Figure 5B).

Finally, anti-aging studies were carried out on the cream produced with both extracts (mixture of *Hypericum perforatum* extract and *Centella asiatica* (L.) extract). Experiments were repeated as 5 times and the results were given as mean ± standard deviation. Comparisons between groups were made with the Kruskal-Wallis and Mann-Whitney tests. According to these tests, differences with a p value of 0.05 and small were considered statistically significant (*p<0.05).

As a result of the test, it was determined that there was a statistical increase in collagen Type I alpha levels at 50 and 100 µg/ml concentrations of anti-aging Herbal Cream compared to the control group. According to these results, Anti-Aging Herbal Cream is an effective anti-aging product (Figure 5C)

When the results in Figure 5 are compared within themselves and with each other, the results when we do not have a cream product are weaker than the results after applying the cream product. In addition, Trial III with both extracts shows better results. Considering the cell line used (ATCC CRL-1635), these are the results that prove the absorption of the active ingredients in the extract from the skin.

CONCLUSION

The aim of the study is to bring together *Hypericum perforatum and Centella asiatica* (L.) plant extracts, which contribute partially to anti-aging studies independently, in the production of a new cosmetic product and with a new formulation. The mixture created is a first both scientifically and commercially. *Hypericum perforatum* and *Centella asiatica* (L.) plant were extracted at different temperatures and with methanol. Active ingredient contents and amounts were determined by HPLC analysis of solid extracts. Asiatic acid and madecasic acid were compared with standards in HPLC spectra. Asiatic acid 9.54 and madecasic acid 6.29. detected in minutes (Figure 3). In addition, the active ingredients of Asiatic acid (0.17%) and Madecasic acid (0.44%) were determined in the *Centella asiatica* (L.) plant (Table 2).

Hypericin could not be detected in the *Hypericum perforatum* (L.) extract in HPLC (Figure.6). For this reason, the processes were continued with *Hypericum perforatum extract* obtained from Aksu Vital company with a hyperforin ratio of 2.5%. The active ingredient ratios in the cream formulation were applied according to the literature. An cream formulation was created with *Hypericum perforatum* and *Centella asiatica* (L.) extracts (Table 1). The ultimate formulation was determined as a result of pH, viscosity, density, protective activity test, and microbial analysis tests.

We wondered if the combination of *Hypericum perforatum* and *Centella asiatica* (L.) showed comparable effects to Hypericum alone or *Centella asiatic* (L.) alone. Cell culture studies were carried out to show the anti-aging effect of the cream formulation. Our data showed that *Hypericum* and *Centella asiatica* (L.) had greater anti-aging effects when combined. This shows us that the combination of the anti-aging effect with *Hypericum perforatum* and *Centella asiatica* (L.) for the cream formulation is possible with lower doses than when they are used individually. It can be concluded that the anti-aging effect is greater than expected based on single factor studies. In in-vitro anti-aging tests, it was revealed that the level of Collagen 1A was higher in the cream formulation where both extracts were used together.

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