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Research Article

Determination of usage potential of *Hypericum perforatum, Hypericum capitatum, Centaurea cyanus* extracts and creams in the cosmetic industry

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ABSTRACT

In recent years, interest in herbal treatments and herbal products has been increasing. Herbal products are preferred because of the properties provided by the secondary metabolites they contain. These products constitute an additional alternative to chemical-added products. Centaury plant is also a popular herb that has been used in the treatment of many diseases for centuries. Secondary metabolites such as hyperforin and hypericin in its structure have provided the plant with various qualities such as antibacterial, antidepressant, antioxidant, etc. properties. These properties of the centaury plant have made it a good alternative for skin treatment. Acne scars and blemishes on the skin over time pose a problem for people. Centaury plant will also be a good option for solving such problems. Generally, various studies are carried out on the healing effects of the *Hypericum perforatum* (Hp) plant on the skin. However, there is limited research on the healing properties of other centaury varieties such as *Hypericum capitatum* (Hc) and *Centaurea cyanus* (Cc). There is no study in the literature on formulating a cream composed of combinations of Hp, Hc and Cc herbs. In this study, plant extracts from three centaury species were obtained by soxhlet extraction methods. The hypericin and hyperforin content of the plant extracts were determined by LC-MS analysis. Creams containing Hc, Hp and Cc extracts and combinations were formulated and antibacterial activities of the creams against *Escherichia coli (E.coli)* and *Staphylococcus aureus* (*S.aureus)* bacteria were determined. As a result of the study, 14 placebo cream containing 5% and 15% centaury extracts and 1 non-centaury extract were produced. It has been concluded that creams containing Cc at 5% and 15% concentrations, Hp at 5% and 15% concentrations, Cc-Hc, Cc-Hp extract in creams can create a potential for use in the cosmetic sector.

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INTRODUCTION

Recently, interest in herbal treatments and products has been increasing rapidly due to the benefits provided by the secondary metabolites of plants. From past to present, the centaury plant has been used both among the public and the health sector to heal various diseases such as wound therapy and antidepressant treatments. Skin problems such as acne and scars pose a problem for people. When the data in the literature are examined, it is seen that the centaury plant will have the potential to be used in the cosmetic industry for the purpose of skin improvement due to its antibacterial, antioxidant and anti-inflammatory properties provided by hypericin and hyperforin metabolites [1].

Hp contains secondary compounds such as hypericin and hyperforin. It has been used to treat wounds, burns and antidepressants for many years [2]. It is seen as a valuable medicine from the ancient Greeks and is used by doctors to heal various diseases such as wound treatment [3]. In a study conducted, it was observed that Hp has anti-inflammatory and antibacterial effects due to its hyperforin component [4]. Based on the antibacterial and anti-inflammatory properties of Hp, a study was conducted on the topical use of Hp oil in the treatment of atopic dermatitis. An extract containing 1.5% hyperforin component was used and the formulated cream was applied to the patients. As a result of the study, it was observed that the cream containing Hp was quite effective compared to the placebo cream [5].

Hc is popularly used to heal the scars caused by acne and wound infections on the skin [6]. Contrary to the studies conducted with Hp, there is a limited number of studies in the literature on Hc and new studies are needed. In a study conducted, it was observed that Hc with methanol extract had an antioxidant effect by clearing free radicals and DNA damage inhibition activity [7].

The use of Cc dates back to ancient times and is used by the public for eye health. It has been observed that it is good for rheumatism pains, skin and skin diseases. There is a limited number of researches on this plant in the literature. Studies are based on the taxonomic classification of the plant and the examination of its metabolic characteristics. Therefore, new studies are needed [8]. In 2017, Arben Haziri et al. tested the extracts of the Cc plant grown in Kosovo on 3 different gram-positive bacteria using the agar disc diffusion method. Ethyl acetate extract with 5mg/mL concentration showed strong antibacterial activity on *E.coli*. It has been stated that the antibacterial activity of CC is due to the presence of various secondary metabolites such as phenols and flavonoids [9].

In this study, Hp, Hc and Cc plants were extracted using the Soxhlet extraction method, and the hypericin and hyperforin ratios in the plant extracts were determined by LC-MS analysis. Then, to determine its potential in the cosmetic sector, the most suitable natural content creams containing these extracts and their combinations were formulated. Their antibacterial activity was determined and the results were evaluated.

MATERIAL AND METHODS

Soxhlet Extraction Method

Dried centaury herbs obtained from the Aegean region were ground using a grinding device. The soxhlet extraction method was preferred as the extraction method [10]. Ethanol was used as solvent. Centaury plants weighing 3 grams were placed in the soxhlet extraction cartridge and placed in the device. Centaury extracts were obtained after the process consisting of immersion, washing and recovery stages [10]. The extracts obtained were stored at room temperature, protected from sunlight.

Liquid Chromatography–Mass Spectrometry (LC–MS) Method

As a result of the literature researches, it was decided to work with the most studied hypericin and hyperforin components among the metabolites of centaury plants [11,12]. In this direction, LC-MS analysis was preferred to determine hypericin and hyperforin components in plant extracts. Two metabolites were analyzed using the Agilent 6530 LC MS-TOF instrument. Hypericin and hyperforin standards were used. Quantitative determinations were made for hypericin and hyperforin analytes by extracting MS / MS in negative mode.

Cream Formulation

Hp, Hc and Cc plant extracts obtained as a result of soxhlet extraction method were used as the active ingredient in the cream formulation. Beeswax, glycerol, glycolic acid, olive oil are used as cream ingredients. It is formulated as an aqueous/lipid type cream. A water bath was used to mix the aqueous and lipid phases homogeneously. Beeswax, olive oil, glycerol and glycolic acid were heated up to 70-75^{0}C in a capsule. This capsule forms the lipid phase (A). Hp, Hc and Cc extracts form the aqueous phase. The extract to be used in another capsule was placed and heated up to 70-75^{0}C (B). Then the aqueous phase was added to the lipid phase and mixed. The mixture, which dissolved homogeneously in each other, was allowed to cool up to 40^{0}C in an agitated water bath [13].

According to the active ingredient amounts; creams with 5% and 15% Hp, Hc and Cc extracts have been formulated in 2 different concentrations. Table 1 gives information about the concentration rates and amounts of creams. In Table 2, detailed information is given about the formulation and amounts of the cream containing 5% and 15% active ingredient. Double and triple combinations of Hp, Hc and Cc extracts and these extracts were created. Including the only extract, 14 different creams in 2 different concentrations were produced, including the double combinations of these extracts Hp-Hc, Hc-Cc, Hp-Cc extracts and includes Hp, Hc and Cc. In addition, a placebo cream without active ingredient was made as a control group.

Table 1. Amounts of cream ingredients

Percentages of Centaury Extract	Amount of Centauera (g)	Base Cream Ingredient Amount (g)	Total Amount(g)
%5	0,5	9,5	10
%15	1,5	8,5	10

Table 2. Amounts of 5% and 15% cream formulation

Material	Amount of 5% cream formulation (g)	Amount of 15% cream formulation
Beeswax	0,5	0,5
Glycerine	0,5	0,5
Glycolic Acid	0,5	0,5
Olive oil	8	7
Plant Extract	0,5	1,5

Antibacterial Activity

The antibacterial activities of the obtained plant extracts and creams were determined by using the agar well diffusion method and disk diffusion method. *S.aureus* and *E.coli* bacteria were used. *E.coli* is a gram-negative bacterium, while *S.aureus* is a gram-positive bacterium. Ampicilin (Amp) antibiotic was used as a control group. The cell revitalization was carried out the day before the bacteria cultivation was done. For this, 20µl of *E.coli* and *S.aureus* bacteria stocked at -80ºC was taken and broth was added to the liquid. Bacteria culture samples were placed in the DATHAN Scientific shaking incubator at 37^{0}C and incubated for 24 hours. After 24 hours of incubation, the optical density of the bacteria taken from the device was measured at 560nm with the Perkin Elmer Lambda 35 branded UV Spectrophotometer. In order to measure the antibacterial activity properly, the bacterial optical density to be cultivated should be between 0.3-0.5*A* [14]. The bacteria were made ready for planting by adding nutrient broth and diluting until the optical density was 0.4. Nutrient agar was used to grow bacterial colonies. Petri dishes were marked so that the antibacterial activity of 4 or 8 cream samples in each agar petri dish was studied. Then, wells were drilled in the compartmentalized points of the agar petri dishes. 100µl of the developed bacterial cultures were taken and planted on an agar medium by smear sowing. After the bacteria cultivation took place, 100 µl cream samples were put into the wells. The prepared media were incubated for 24 hours at 37^{0}C. Following 24 hours of incubation, the inhibition diameters of the samples taken from the incubator in the *S.aureus* and *E.coli* cultures were measured. This process was repeated 3 times for each sample. The calculation was made by taking the average of the results.

RESULTS AND DISCUSSION

LC–MS Results

In the results of LC / MS analysis, it was concluded that the amount of hypericin and hyperforin components in Hp extract was higher than the amount of hypericin and hyperforin in Hc and Cc plant (Table 3).

Cream Formulation Results

As a result of cream formulations; A total of 15 creams were created, including 14 creams containing 5%, 15% Hp, Cc, Hc extract and their combinations, and placebo creams without plant extracts. The cream samples produced are shown in Figure 1.

The pH value of the creams was examined. It has been determined that the pH values of the creams are in the range of 6-7. Color, odour, pH and viscosity properties of the

Table 3. Results of determination of hypericin and hyperforin of Centaury extracts

	Hypericin Amount	Hyperforin Amount
Hypericum perforatum	153±0.005 ng mL-1	154.9±0.018 ng mL-1
Centaurea cyanus	12.5±5.2 ng mL-1	43.2±0.7 ng mL-1
Hypericum capitatum	22.4±5.3 ng mL-1	59.5 ng mL-1

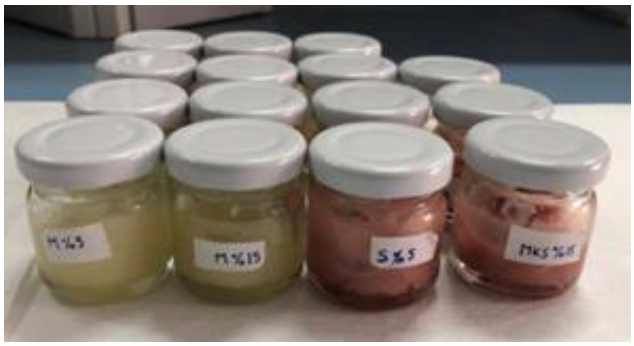

Figure 1. Examples of creams produced.

creams in the 1, 2, 6 and 12th months after obtaining were examined. It was observed that the creams are stable in terms of color, odor, pH and viscosity at the end of this period.

Antibacterial Activity Results

By looking at the antibacterial activities of the extracts and creams produced, the potential for the using extracts and creams in wound care was evaluated. In Figure 2, the inhibition zone formation effects of Hp, Hc and Cc extracts on *E. coli* and *S. aureus* cultures are shown. In Figures 3, 4, 5 and 6 the inhibition zone formation effects of the creams that include Hp, Hc and Cc extract and combinations on *E.coli* and *S.aureus* cultures were shown. The inhibition diameters of the extracts and creams in *E.coli* and *S.aureus* cultured media were measured. According to the results of agar well diffusion method and disk diffusion method;

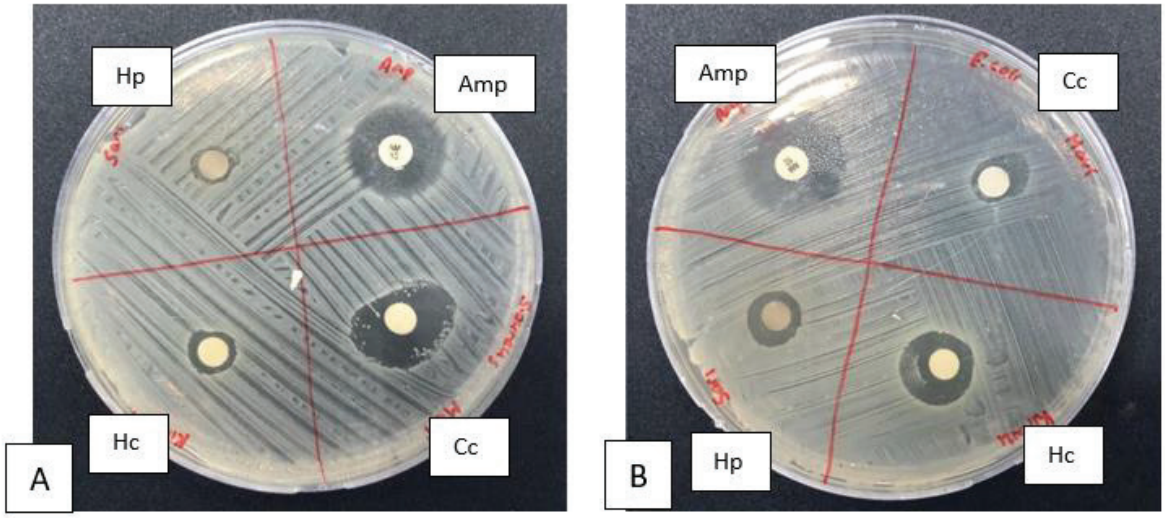

Figure 2. (A) Effect of liquid extracts on *S. aureus* (B) Effect of liquid extracts on *E. coli*.

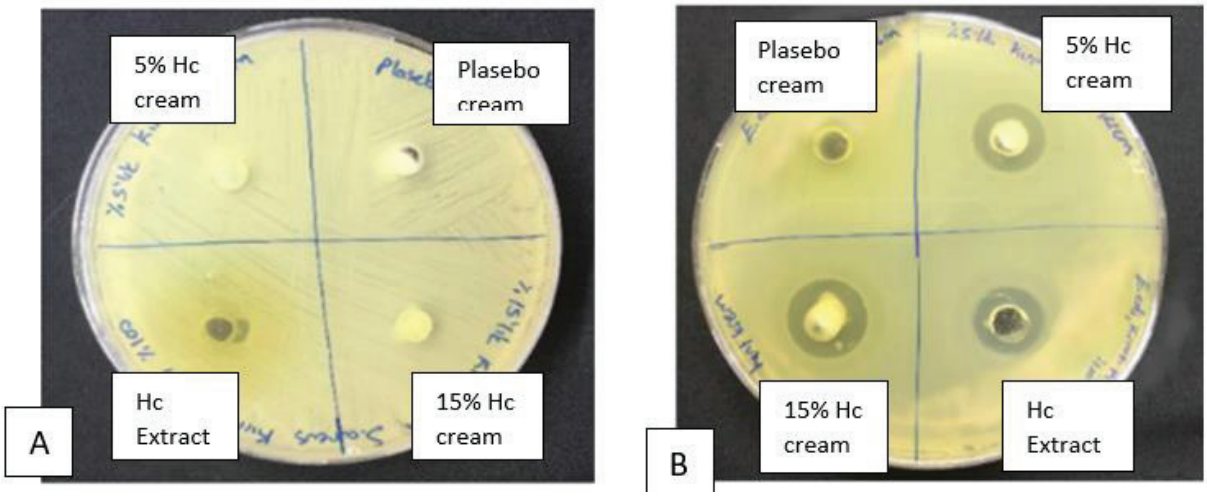

Figure 3. (A) Effect of Hc extract and creams containing extract and placebo cream on *S. aureus* (B) Effect of Hc extract and creams containing extract and placebo cream on *E. coli*.

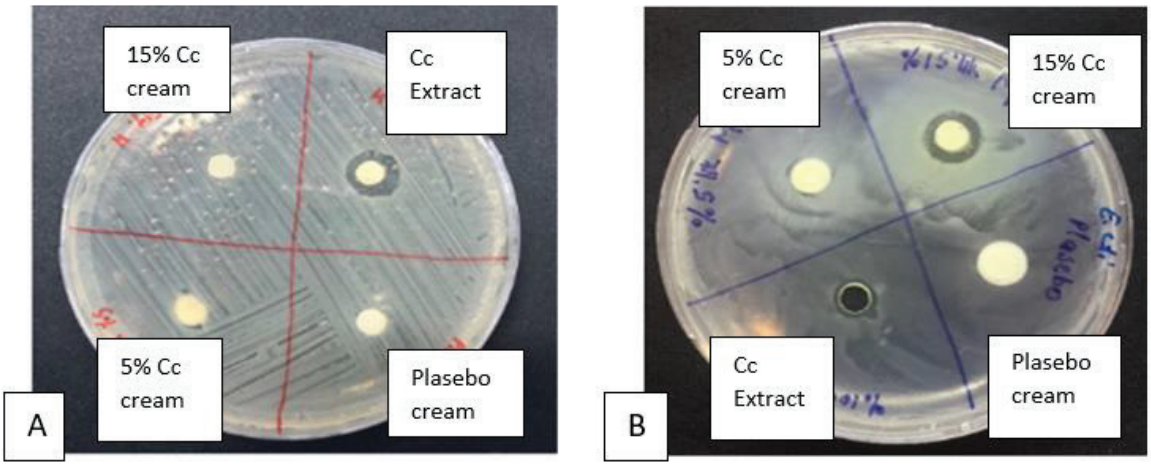

Figure 4. (A) Effect of creams containing Cc extract and placebo cream on *S. aureus* (B) Effect of creams containing Cc extract and placebo cream on *E. coli*

it was observed that all cantaury species exhibited different antibacterial activities at certain rates against *S.aureus* and *E.coli* bacteria, and the effects they exhibited differed according to the bacteria species.

It was observed in Figure 2 (A) that Cc extract has a higher antibacterial effect on *S.aureus* bacteria against Hp and Hc extracts. In addition, Cc extract has a higher effect compared to the control group Amp disc. It was observed in Figure 2 (B) that Hc extract has a higher effect against *E.coli* bacteria compared to Hp and Cc extracts. It has been determined that Cc has a higher antimicrobial effect on Gram-positive bacteria and Hc has a higher antimicrobial effect on Gram-negative bacteria.

In Figure 3, it was observed that Hc extract and 5%, 15% creams have more effective antibacterial activity in *E.coli* than *S.aureus*.

In Figure 4, it was observed that Cc extract has a higher antibacterial effect on *S.aureus* and 5%, 15% creams have more effective antibacterial activity in *E.coli* than *S.aureus*.

In Figure 5 (A), it was observed that Hp extract formed an inhibition zone in the medium containing *S.aureus* bacteria, but 5% and 15% creams were not effective. In Figure 5 (B), it was observed that the Hp extract formed an inhibition zone in the medium containing *E.coli* bacteria, the 15% cream formed an inhibition zone with a smaller diameter, and the 5% cream did not affect.

When Figure 6 (A) is examined, it was observed that creams containing 5% and 15% Cc-Hc extract, 5% Cc-Hp and 5% Hp-Hc extract created an inhibition zone in a medium containing *S.aureus* bacteria. When Figure 6 (B) is examined, it is concluded that creams containing 5% and

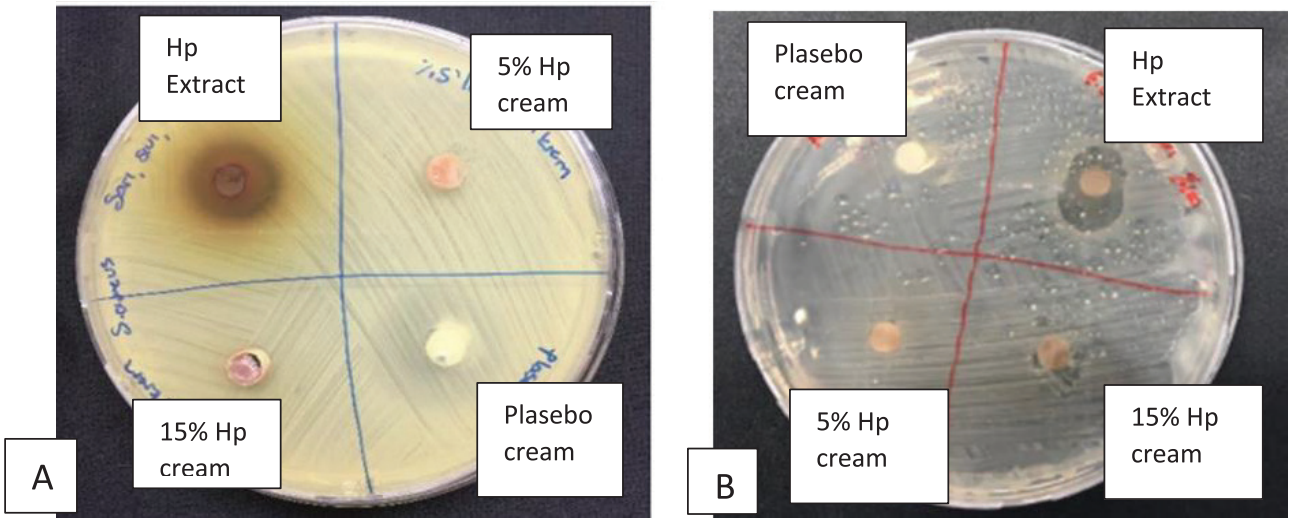

Figure 5. (A) Effect of Hp extract and creams containing extract and placebo cream on *S. aureus* (B) Effect of Hp extract and creams containing extract and placebo cream on *E.coli*.

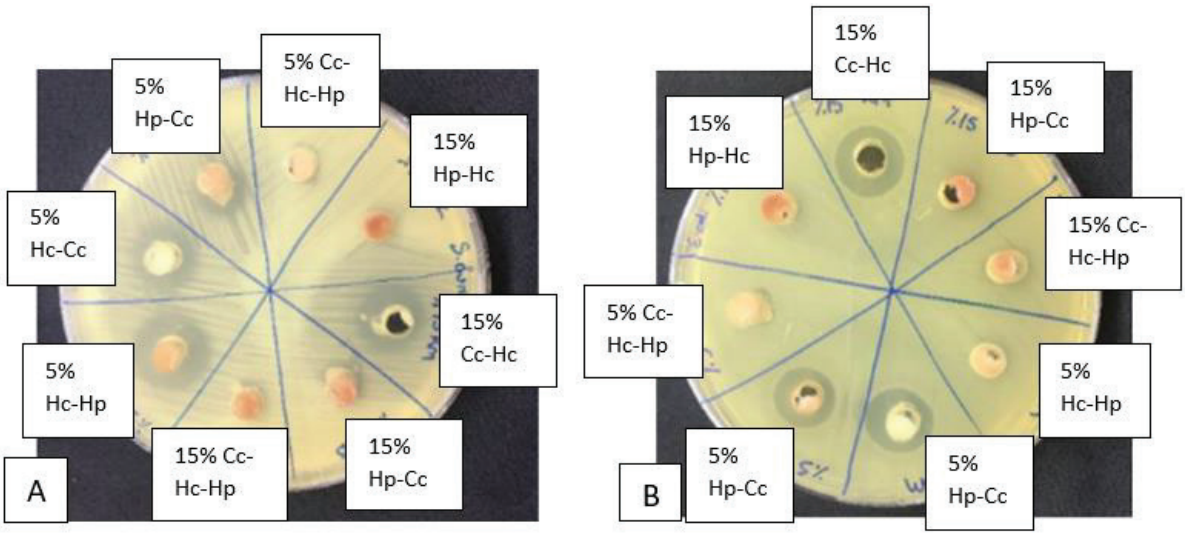

Figure 6. (A) The effect of creams containing Hp-Hc, Cc-Hp, Hc-Cc and Hp-Hc-Cc extracts and placebo cream on *S.aureus* (B) The effect of creams containing Hp-Cc, Hc-Cc, Hp-Hc and Hp-Hc-Cc extracts and placebo cream on *E.coli.*

15% Hc-Cc and creams containing 5% Cc-Hp extract have antibacterial activity on *E. coli*.

In addition, it has been shown that the antimicrobial effects of plant extracts differ according to bacterial species such as Gram-positive and Gram-negative bacteria. Amp the control group in this study; except for the antibacterial effect of Cc extract on *S.aureus* bacteria; it showed higher inhibitory effect against Hc, Cc and Hp extracts, creams and gram-negative and gram-positive bacteria tested at all concentrations.

At the end of the incubation, inhibition zone diameters formed in media containing *E.coli* and *S.aureus* were measured. Results are indicated in Table 4 and Table 5.

Table 4. Inhibition zone diameter measurement results of Hp, Hc and Cc extracts

	E.coli	***S.aures***
***Centaurea cyanus* extract**	8.2mm±0.5	16.9mm±0.8
***Hypericum perforatum* extract**	12mm±0.7	7.4mm±0.7
***Hypericum capitatum* extract**	9.7mm±0.8	6.9mm±0.6
Ampicilin	17.1mm±0.6	15.8mm±0.8

Table 5. Inhibition zone diameter measurement results of creams containing Hp, Hc and Cc extract, Hc-Cc, Hp-Cc, Hp-Hc and Hp-Hc-Cc extracts

	E.coli		***S.aureus***	
	Creams containing 15% extract	**Creams containing 5% extract**	**Creams containing 15% extract**	**Creams containing 15% extract**
Hypericum perforatum	-	-	-	-
Hypericum capitatum	12.1mm±0.5	13.2mm±0.9	-	-
Centaurea cyanus	10.5mm±0.7	2mm±0.6	-	-
Hypericum capitatum-Centaurea cyanus	14.2mm±0.8	12.3mm±0.5	12mm±0.6	9.7mm±0.7
Hypericum perforatum-Centaurea cyanus	-	10.7mm±0.6	-	10mm±0.5
Hypericum perforatum-Hypericum capitatum	-	-	-	12mm±0.7
***Hypericum perforatum-Hypericum capitatum-Centaurea cyanus* extracts**	-	-	-	-

It is thought that the reason for the high antibacterial activity of Cc and Hc compared to Hp is due to the fact that they contain different metabolites compared to Hp. Therefore, although hypericin and hyperforin metabolites in Hp are high, their antibacterial activity was found to be low [9].

CONCLUSIONS

Hp, Hc and Cc plants collected from our country were extracted using the soxhlet extraction method. Hypericin and hyperforin components of the extracts were determined using the LC / MS analysis method. It was concluded that the values of hypericin and hyperforin components in Hp extract were higher than the values of Hc and Cc. Beeswax, olive oil, glycerol, glycolic acid materials are preferred for use in a cream formulation. 14 pieces of centaury extract containing 5% and 15% centaury extract and one placebo cream with a control group without centaury extract were produced. Antibacterial activities of liquid extracts and cream samples using agar well diffusion and disk diffusion method were tested using *E.coli* and *S.aureus* bacteria. The diameters of inhibition that occurred were measured. As a result of these measurements, it was observed that the 2-in-1 combination creams containing Cc and Hc extract have more effective antibacterial activity compared to creams consisting of Hp and other combinations. It appears that the cream containing the extract of Cc and Hc at a concentration of 15% has a higher potential than the cream at a 5% concentration. Additionally, creams containing 5% and 15% Cc-Hc and 5% and 15% Cc-Hp extract were also observed antibacterial effects.

As a result, it was concluded that creams containing Hc and Cc extracts at 5% and 15% concentrations of Cc, 5% and 15% of Hc, Cc-Hc, Cc-Hp extract can create a potential for use in the cosmetic sector has been reached.

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AUTHORSHIP CONTRIBUTIONS

Authors equally contributed to this work.

DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS

There are no ethical issues with the publication of this manuscript.

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