Current Research in Pharmaceutical Sciences
Available online at www.crpsonline.com

ISSN: 2250 – 2688

Received: 07/08/2024
Revised: 22/08/2024
Accepted: 09/12/2024
Published: 08/01/2025

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DOI: 10.24092/CRPS.2024.140401

Website: www.crpsonline.com

Quick Response Code:

NEW FORMULATED VANISHING SKIN CREAM CONTAINING SRI LANKAN RAW HONEY VS MARKETED CREAMS WITH HONEY: A COMPARATIVE STUDY

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ABSTRACT

Aims: Cosmetics containing natural ingredients have become more popular nowadays due to their ability to beautify and treatment of skin ailments. Honey is an organic natural substance with various benefits and traditional uses. The objective of this study was to formulate a new vanishing skin cream incorporating Sri Lankan raw honey and to compare it with creams already available in the market.

Methods: Three different bee honey concentrations of Sri Lankan origin were incorporated into vanishing cream bases to produce three new cosmetic creams (F1-12.5% w/w, F2-18.75% w/w, F3-25% w/w). They were compared with three different commercially available cosmetic creams (C1-local honey (Honey day cream/Fairness day moisturizer, Ovi, Sri Lanka), C2-foreign honey (Almond milk and honey cream, Body Shop, USA), C3-foreign (Milk and honey gold nourishing hand and body cream, Oriflame, Sweden) for stability, antibacterial activity (agar well diffusion assay), and antioxidant activity (DPPH assay).

Results: All cream formulations were stable at room temperature (25°C) and produced inhibitory zones against *Escherichia coli* and *Staphylococcus aureus*. DPPH assay revealed that all cream samples have antioxidant activity. The pH of laboratory-made cream formulations containing Sri Lankan raw honey was compatible to be used for topical application. C1 cream showed the highest antioxidant and antibacterial activity compared to all other creams. Antioxidant and antibacterial activities increased with increasing honey concentrations for formulated creams.

Conclusions: It is concluded that the laboratory-made new cream formulations containing Sri Lankan raw honey were compatible to be used for topical application compared with the creams already in the market and have future potential as a cosmeceutical.

Keywords: Sri Lankan raw honey; cosmetic creams; antibacterial activity; antioxidant activity.

1 INTRODUCTION

Cosmeceuticals have become very popular due to their medicinal properties along with beauty-enhancing effects[1]. The difference between cosmeceuticals and pharmaceuticals is important for contemporary dermatological practices. Pharmaceuticals are used to change the skin and thereby protect the skin from pathologic conditions. Cosmeceuticals are the hybrid between cosmetics and pharmaceuticals[2]. The benefits of cosmeceuticals depend on the active ingredients. The vehicle of the formulation is important to determine the number of active ingredients that penetrate the epidermis to exert the effects[3].

Cosmeceuticals have various indications depending on the target condition using various types of ingredients; natural as well as synthetic such as skin lightening, moisturizing, anti-wrinkling, scar-reducing, antioxidants, antimicrobial, and hair strengthening[4]. Cosmetics can also be identified as

products that are used to clean, perfume, beautify, odorant, and produce well-being of the skin[5]. And they are not affecting the structure and function of the skin. The cosmetic industry is focusing on developing cosmeceuticals because of their high value nowadays[6].

Cosmeceuticals containing synthetic compounds such as acyclovir, triamcinolone, calcipotriene, and mometasone give fairness to the skin but they produce side effects such as itching and several allergic conditions. Cosmeceuticals with natural components give fairness to the skin without side effects[7]. In addition, cosmeceuticals containing natural ingredients have various properties such as antioxidant, anti-inflammatory, antibacterial, and antiseptic properties[8].

Creams are semi-solid emulsions. They are applied to the skin or mucous membranes. Vanishing cream is o/w emulsion with high patient acceptability because of its less oily texture and since it leaves nothing on the skin after application. Honey plays a role in wound healing. It is attributed to its antibacterial activity, immunomodulatory properties, and its ability to maintain moist wound conditions. Due to high viscosity, honey provides a protective barrier to prevent infections[9]. In many of the honey types, antimicrobial activity is exhibited through the enzymatic production of hydrogen peroxide. The low pH and high sugar content also play roles in antibacterial activities[10]. Honey has shown antibacterial activity against *Enterobacter erogen*, *Staphylococcus aureus*, *Salmonella zyphimurium*, and *E.coli*[11].

The objective of this study was to formulate a new vanishing skin cream incorporating Sri Lankan raw honey and to compare it with creams already available in the market. As such in the current study, three different bee honey concentrations of Sri Lankan origin were incorporated into vanishing cream bases to produce three new cosmetic creams (F1-12.5% w/w, F2-18.75% w/w, F3-25% w/w). Then, they were compared with three different commercially available cosmetic creams (C1-local honey, C2-foreign honey, C3-foreign honey) for stability, antibacterial activity (agar well diffusion assay), and antioxidant activity (DPPH assay).

2 MATERIALS AND METHODS

2.1 Preparation of cream samples

Bee honey samples of Apis cerana species were collected from Sabaragamuwa province in Sri Lanka from wild colonies that are naturally available in small forest areas and was used to prepare three cream formulations by incorporating different weights (Table 1). The oil phase and aqueous phase were prepared separately by heating the respective constituents (oil phase: stearic acid and cetostearyl alcohol, aqueous phase: glycerine, potassium hydroxide, phenoxy ethanol, citric acid and distilled water) up to 65°C±5°C using a hot plate. The raw honey mixture was added when the aqueous phase was cooled to 40°C and stirred well then, the oil phase (40°C) was incorporated into the aqueous phase while stirring.

2.2 Stability analysis of the cream

The stability of the formulated creams and commercial creams was evaluated through accelerated stability testing (Visual observation, Centrifuge testing, Freeze-thaw test, and Characterization (pH, organoleptic properties and emulsion type determination))[12,9].

Table 1. Ingredients and their percentages used in the preparation of cream formulations.

Ingredients	Placebo (Base)	Form.1 (F1)	Form.2 (F2)	Form.3 (F3)
Raw bee honey mixture	-	12.50%	18.75%	25%
Stearic acid	13%	13%	13%	13%
Cetostearyl Alcohol	3.30%	3.30%	3.30%	3.30%
Glycerin	10%	10%	10%	10%
Potassium hydroxide	0.50%	0.50%	0.50%	0.50%
Phenoxy ethanol	0.50%	0.50%	0.50%	0.50%
Citric acid	0.40%	0.40%	0.40%	0.40%
Distilled water	72.30%	59.80%	53.55%	47.30%

2.2.1 Visual observation

Each cream formulation (5 g) was kept at different storage conditions (8°C - refrig-erator, 25°C - room temperature, 40°C - hot air oven) and characteristics of creams (creaming, phase separation, sedimentation, coalescence, flocculation, color change in top layer and surface dryness) were observed at each storage conditions on 1st ,3rd ,7th ,15th and 25th-day [12].

2.2.2 Centrifuge testing

Each cream formulation (5 g) was placed in a stoppered centrifugal tube and was centrifuged (PLC- 036H,Taiwan, 2016) at 1200 rpm for 3 minutes at 25°C on the 1st, 3rd, 7th, 15th, and 25th day. Then, the cream formulations were observed for phase separation, or other physical changes [9].

2.2.3 Freeze thaw test

Each cream formulation (5 g) was kept first in a freezer (SAMSUNG brand, China) at -5°C for 24 hours, and then the

frozen samples were left at room temperature for 24 hours to thaw. This cycle was repeated two times. After each cycle, the creams were evaluated for phase separation. However, if a phase separation was observed after the first freeze-thaw cycle, the test was not continued [12].

2.2.4 pH determination

Each cream formulation (0.5 g) was weighed and dissolved properly (with no visible particles) in 50 ml of distilled water and pH was measured in creams that were at different storage conditions (8℃ - refrigerator (SAMSUNG brand, China), 25℃ - room temperature, 40℃ - hot air oven) on 1st, 3rd, 7th, 15th and 25th day respectively after preparing the formulations [9].

2.2.5 Organoleptic properties

The appearance (color, odor and texture) was evaluated in creams that were at different storage conditions (8℃ - refrigerator, 25℃ - room temperature, 40℃ - hot air oven) on 1st, 3rd, 7th, 15th and 25th day respectively after preparing the formulations [12].

2.2.6 Determination of the emulsion type

In this test, around 0.1 g of the cream was taken, and it was mixed with methylene blue(0.1ml). Then a small portion of the mixture was placed on a clean and dry glass slide and spread over the slide using narrow edge of another slide to make a thin film [12]. The thin film was covered with a cover slip and observed under the light microscope (40x objective).

2.3 Determination of antimicrobial activity

The antibacterial activity against *E. coli* and *S. aureus* was determined using an agar well diffusion assay. Bacterial suspensions were prepared by dissolving 24-hour *E. coli* and *S. aureus* cultures in saline and standardized using the 0.5 McFarland standard. Three wells were punched in three divided parts of an agar plate with an 8 mm diameter sterile cork-borer. Agar solution (0.1 ml) was added to each well to seal the well. After it solidified, wells were filled with 0.1 g of each cream formulation, 0.1 ml of sterile distilled water (negative control), and 0.1 ml of 0.025 mg/mL Gentamycin solution (positive control). Plates were incubated at 37°C in an inverted position for 24 hours. The mean diameter of the zone of inhibition (in mm) was observed[13,14].

2.4 Determination of antioxidant activity

DPPH assay[15] was used to determine the antioxidant activity of the creams. F1(6.4000g), F2(6.4000g), F3(6.4000g), C1(0.4000g), C2(6.4000g) and C3(6.4000g) creams were weighted using analytical balance (VWR brand, Italy) and thoroughly mixed with methanol (50ml). The mixtures were filtered using filter paper and the filtrates were considered as stock solutions of each sample. Respective solutions were used to prepare concentration series of each cream sample (8 mg/mL, 4 mg/mL, 2 mg/mL,1 mg/mL, 0.5 mg/mL, 0.25 mg/mL). 0.1mM DPPH solution was used for the evaluation of antioxidants and ascorbic acid was used as the positive control.

The test samples (1ml) were mixed with freshly prepared DPPH solution (1ml). The sample blanks were prepared using each cream sample solution (1ml) that was mixed with methanol (1ml). Negative control was prepared using methanol(1ml) that was mixed with DPPH(1ml). The negative control blank was methanol(2ml). Prepared samples were kept in the dark for 30 mins. The absorbance was measured at 517 nm using a UV spectrophotometer (GENESYS 10s UV-Vis, ThermoFisher, 2008). The scavenging activity of the sample against the stable DPPH was calculated using the following equation[15,16].

$$\text{Scavenging activity \%} = [(A - B) / A] \times 100$$

Where,

A= Absorbance value for the negative control and
B= Absorbance value for test sample

3 RESULTS

3. 1 Stability test

3.1.1 Visual observation

All cream formulations that were kept at 8°C and 25ºC were stable throughout the 25 days however visual changes were observed in cream formulations kept at 40°C. The base formulation was stable throughout 25 days study period in all three temperatures (Figure 1).

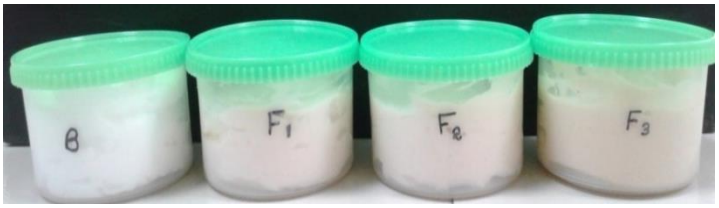

Figure 1. Appearance of cream formulations after preparation.

3.1.2 Centrifuge testing

The Base and the cream formulations F1, F2, C1 and C3 did not show phase separation or any other physical changes under centrifugal force throughout the 25-day study period. However, in F3 cream formulation a small amount of honey separated from the formulation from 15th day onwards. Similarly, in C2 cream formulation, a small aqueous layer was observed from 3rd day onwards (Table 3).

3.1.3 Freeze-thaw test

Phase separation or any physical changes were not observed in all cream formulations after two cycles.

3.2 Characterization

3.2.1 pH of cream formulations at different temperatures

The pH of all cream formulations kept in 8^{O}C was lower than its initial values. All cream formulations showed significant pH variation (B p - 3.93E-07, F1 p - 2.34E-07, F2 p - 0.0013, F3 p - 0.0095, C1 p - 5.57E-08, C2 p - 4.24E-08 and C3 p - 3.8E-08) with respect to time.

By the 25th day, the pH of all cream formulations kept at 40°C was lower than its initial values and showed significant pH variation (B p -1.64E-07, F_1 p -7.08E-07, F_2 p - 2.44E-05, F_3 p - 0.0011, C_1 p - 1.01E-09, C_2 p - 2.49E-06 and C_3 p - 5.57E-08).

3.2.2 Organoleptic properties

Some cream formulations (C1 and C3) including the base did not show any color change and their characteristic color continued to appear the same at different temperature conditions throughout the 25-day study period. A dark brown color was observed in F1, F2, F3 cream formulations at 40℃ on the 15th day and afterward and a yellow color was observed in C2 cream at 40℃ on the 25th day (Table 5).

3.2.3 Odour

Cream formulations (F1, F2, F3, C1, C2 and C3) which were kept at different temperature conditions had a pleasant odor during the 25 days and no characteristic odor was observed in the base formulation from the beginning.

3.2.4 Texture

A moderately fine texture was observed in the base formulation at different temperature conditions and in F1, F2 and F3 cream formulations at 8°C and 25℃ throughout the 25-day study period while a very fine texture was viewed in marketed cream formulation (C1, C2 and C3) in the same conditions.

A moderately fine texture was observed in F1, F2 and F3 cream formulations kept at 40℃ until 7th day however coarse texture was observed on these formulations from the 15th day onwards.

A very fine texture was observed in C1 and C2 cream formulations kept at 40℃ until 7th day but a coarse texture was observed on the 15th day and afterward. A very fine texture was viewed in C3 cream formulation kept at 40℃ until the 15th day but a coarse texture was observed on the 25th day.

3.2.5 Emulsion type

Colorless globules dispersed in a blue color phase in all cream formulations. Since methylene blue is water soluble, these globules are oil droplets and therefore these formulations were confirmed to be o/w emulsions.

3.3 Antimicrobial activity

The inhibition zone diameters for the laboratory-made cream samples increased with the increasing honey concentration in F1, F2 and F3. All the commercial creams showed higher zones of inhibition than the laboratory-made creams against *S. aureus*. The highest zone was observed in C1 cream with local honey against *E. coli* as well as *S. aureus*. However, there was no significant difference between the zones of inhibition observed between C1 and all laboratory-made creams. Cream base did not show any zone of inhibition against both *E. coli* and *S. aureus* (Figure 3).

3.4 Antioxidant activity

IC50 values of the laboratory made cream formulations decreased with increasing of honey concentration. C1 cream with local honey showed the lowest IC50 value compared with all the other cream samples while C3 cream showed the highest IC50 value. Therefore, C1 cream has the highest antioxidant activity compared to all the other cream samples whereas C3 cream has the lowest activity. Interestingly antioxidant activity of F1, F2 and F3 was higher than the commercial creams C2 and C3 (table 5).

4 DISCUSSION

In this study, stability, characterization, antibacterial activity (against *E. coli* and *S. aureus)* and antioxidant activity of laboratory-made new cream formulations with Sri Lankan honey were compared with commercially available creams containing honey.

Vanishing Creams are an oil-based emulsion. They leave behind a thin, imperceptible oil-free layer on the skin's surface after application. Normally, they are utilized to increase adhesion and holding power to the skin. In this study, the vanishing cream was prepared by incorporating bee honey as the active ingredient. Stearic acid provides the consistency of the cream and pearlescent property to the cream. Cetostearyl alcohol is used to soften the skin and to thicken and stabilize the cream. Potassium hydroxide gives fine texture and consistency without harshness. Glycerin is a humectant that prevents the drying of the cream[17]. Phenoxy ethanol is a preservative that prevents the deterioration of the cream by bacteria. Citric acid exfoliates the dead skin cells in the upper layer of the skin and helps to clean the pores, smooth the skin and provide even skin tone.

The most important consideration with respect to pharmaceutical products is the stability of the end product. The formulated creams were subjected to both real-time stability and

Table 2. The visual observation of cream formulations at different temperature

				8°C							25°C						40°C			
	B	**F_1**	**F_2**	**F_3**	**C_1**	**C_2**	**C_3**	**B**	**F_1**	**F_2**	**F_3**	**C_1**	**C_2**	**C_3**	**B**	**F_1**	**F_2**	**F_3**	**C_1**	**C_2**	**C_3**
1st	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3rd	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
7th	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
15^{th}	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	C	C	C	R	R	S
25^{th}	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	C	C	C	R	R	R

Table 3. Phase separation after subjecting to centrifugation

Days			**Phase separation**				
	B	**F_1**	**F_2**	**F_3**	**C_1**	**C_2**	**C_3**
1st	N	N	N	N	N	N	N
3rd	N	N	N	N	N	N/Aq	N
7th	N	N	N	N	N	N/Aq	N
15^{th}	N	N	N	H	N	N/Aq	N
25^{th}	N	N	N	H	N	N/Aq	N

Table 4. Color of cream formulations at different temperatures

Days	**B**			**F_1**			**F_2**			**F_3**			**C_1**			**C_2**			**C_3**		
	8	**25**	**40**	**8**	**25**	**40**	**8**	**25**	**40**	**8**	**25**	**40**	**8**	**25**	**40**	**8**	**25**	**40**	**8**	**25**	**40**
1^{st}	W	W	W	B	B	B	B	B	B	B	B	B	P	P	P	W	W	W	Y	Y	Y
3^{rd}	W	W	W	B	B	B	B	B	B	B	B	B	P	P	P	W	W	W	Y	Y	Y
7^{th}	W	W	W	B	B	B	B	B	B	B	B	B	P	P	P	W	W	W	Y	Y	Y
15^{th}	W	W	W	B	B	D	B	B	D	B	B	D	P	P	P	W	W	W	Y	Y	Y
25^{th}	W	W	W	B	B	D	B	B	D	B	B	D	P	P	P	W	W	Y	Y	Y	Y

Table 5. IC50 values obtained for cream formulations

Sample	IC50 Value
F1	90.37
F2	60.62
F3	54.52
C1	3.88
C2	144.39
C3	271.52
Ascorbic Acid	1.97

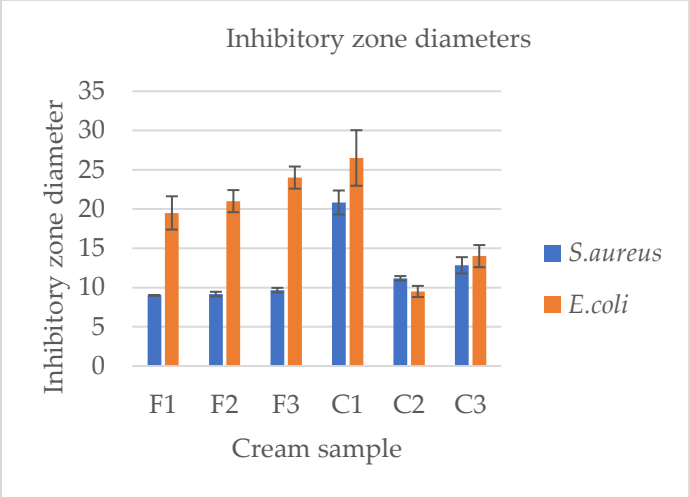

Figure 2. Inhibitory zone diameters

accelerated stability testing to determine the shelf-life and storage conditions of the cream and to select the best formulation among them[9]. Accelerated stability studies are conducted in short period of time. Physicochemical parameters of creams are good indicators of their quality and stability[18]. Emulsion stability can mainly be identified by retaining its physical characteristics like odor, color and appearance[19].

Both laboratory made cream formulations (B, F1, F2 and F3) and marketed creams (C1, C2 and C3) kept at 8℃ and 25℃ were stable. But F1, F2 and F3 kept at 40℃ showed a color change on the top layer of the emulsion and dryness on the surface on the 15th day and afterward. The reason for this may be due to the absorption of moisture from the surface of the emulsion. The marketed cream formulations kept at 40℃ showed some instability characteristics on the 15th day and afterward. When the overall results of visual observation are considered, it can be concluded that all cream formulations were visually stable at room temperature (25℃).

No phase separation on centrifugation was seen in most of the creams (B, F1, F2, C1 and C3) kept at 25℃ during the study period. The proper homogenization rate in the emulsion formulation prevents the creams from breaking during stressful conditions. Although the F3 cream formulation did not show a complete phase separation, a small amount of honey was separated from the cream on the 15th day and afterward. The high honey concentration could be the reason for this instability.

Freeze-thaw is an accelerated stability test that is conducted by exposing the cream formulation to harsh thermal stress just after formulation to evaluate the stability after extreme thermal stress. All the cream formulations were stable during the freeze thaw tests.

The pH of cream formulations intended for application to the skin should be close to the pH range between 4.5-6.5. In this study, the initial pH range of laboratory-made cream formulations was between 5.0 and 6.5 which is appropriate for the human skin. However, it was observed that the initial pH of marketed creams was more than 7. In addition to honey, other plant and animal substances contained in these creams can increase their pH value. Honey has a predominantly acidic nature, mostly due to the content of gluconic acid, formed as a result of the activity of glucose oxidase. Therefore, the initial pH of F1, F2 and F3 (pH - 5.93±0.338) cream formulations was lower than the initial pH of the base formulation (pH – 6.45±0.181). Also, the pH of the laboratory-made creams (except base) decreased as the concentration of honey increased.

The agar well diffusion method was used to evaluate the antibacterial activity of the cream samples in the current study. Research has been conducted on manuka honey which revealed that manuka honey has antibacterial activity against *E.coli* and *S. aureus*[13]. The laboratory-made creams have comparatively high antibacterial activity against *E. coli* but very low antibacterial activity against *S. aureus* compared to the commercial creams. Results revealed that when the honey concentration is increased, both the antibacterial activity and the antioxidant activity are increased. It was noted that laboratory-made creams have higher antioxidant activity than two of the commercially available creams, C2 and C3.

The results indicate that all cream formulations tested were stable at room temperature (25℃) based on parameters such as visual observation, centrifugal force, and organoleptic evaluation. The pH of laboratory-made cream formulations containing Sri Lankan raw honey was compatible with topical application.

5 CONCLUSION

It is finally concluded that laboratory-made new cream formulations containing Sri Lankan raw honey were compatible with topical application compared with the creams already in the market and have future potential as a cosmeceutical.

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