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Original Article

Harnessing Beet Root Pigments as Natural Colorant for a Glossy Herbal Lipstick: Formulation and Evaluation

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ABSTRACT

Background: Lipstick is a solid cosmetic designed to impart vibrant colour, hydration, and textural enhancement to the lips. With a growing consumer shift toward natural products. Interest in plant-based colorants and natural emulsifiers has surged. Herbal lipsticks offer enhanced biocompatibility, reduced dermal irritancy. It is available in finishes such as matte, glossy, satin, and metallic, glossy but has unique formulation challenges due to water incorporation. This study aimed to develop and evaluate herbal lipsticks using *Beta vulgaris* extract as a natural chromophore and diverse natural emulsifiers. **Materials and Methods:** Four Formulations (F1-F4) were crafted using beeswax, castor oil, coconut oil, beetroot extract, and natural emulsifiers such as beeswax (E1), lecithin (E2), lanolin (E3), and candelilla wax (E4). The process included solvent-extraction, emulsification, wax melting, molding, and surface flaming. Each formulation was rigorously evaluated for solubility, melting point, pH, colour intensity, mechanical strength, surface anomalies, fragrance retention, water resistance, thixotropy, aging stability, antimicrobial activity, skin compatibility, phytochemical composition, and DSC. **Results:** All four herbal lipstick Formulations (F1-F4) showed good solubility in organic solvents and maintained a pH range 5.5 to 6.8. F2 had the highest colour intensity and best spreadability, while F1 showed superior mechanical strength and thermal stability. All formulations were microbiologically safe, stable, and exhibited favorable thixotropic behavior. Phytochemical screening confirmed the presence of flavonoids and phenols. **Conclusion:** F2 emerged as the most effective, attributed to lecithin's emulsification efficiency, offering a promising, elegant natural alternative to conventional lipsticks.

Keywords: Beet root, Colour intensity, Dispersion, DSC, Herbal lipstick, Surface anomalies.

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INTRODUCTION

Some recent research suggests that lips serve as a visible indicator of a woman's fertility. Studies in evolutionary psychology indicate that red lips are associated with higher estrogen levels, signaling health and fertility (Science Note, 2005). Lipstick is a cosmetic preparation that contains pigments, oils, waxes, and emollients to give the lips colour and texture. In Britain, it is commonly referred as "lippy" (Bhagyasree *et al.*, 2024). The colouring of lips is not a new concept. In ancient India, orthodox women used to *chew* pan *a* mixture of crushed areca nut, betel leaves, spices, and lime to add colour to their lips (Natnoo, 2018). This practice can still be observed in Indian culture today, especially during festivals such as Holi and Raja (Iftikhar, 2010).

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Today, lipsticks are available in various forms, including transparent lipstick, liquid lipstick, lip rouge, lip jelly, lip salve, lip gloss, lip balm, and lip pencils (Sharma, 2008). However, the main concern remains the use of synthetic colours. In the United States, colour additives in cosmetics do not require FDA approval before being marketed. However, in October 2007, the Campaign for Safe Cosmetics reported the presence of lead in several commercially available lipsticks. In response, the FDA issued draft guidance in December 2016, recommending that cosmetic lip products such as lipsticks, glosses, and liners should not contain more than 10 parts per million (ppm) of lead as an impurity. This limit aligns with standards set for food products due to the likelihood of incidental ingestion (U.S. Food and Drug Administration [FDA], 2022). Furthermore, some research articles have highlighted the potential carcinogenic effects of artificial colours (Rasheed *et al.*, 2020). This concern has led to increased demand for herbal lipsticks (Jain *et al.*, 2022).

Despite their growing popularity, herbal lipsticks present several formulation challenges, including inconsistent shades, shorter shelf life, low melting points, and a higher risk of microbial contamination. These issues often arise due to the instability of natural ingredients and the absence of effective binding and stabilizing agents. To address these limitations, the present study introduces a novel approach by incorporating various emulsifying agents such as beeswax, lecithin, lanolin, and candelilla wax.

An emulsifier is a substance that helps to mix two immiscible liquids, like oil and water. Normally, these liquids separate as they are not thermodynamically stable, but an emulsifier enables them to blend uniformly and increase the stability of the formulation (Arian Velayati and Alireza Nouri, 2020). Emulsifiers have two parts, one that attracts water and another that attracts oil. The water-loving part points toward the water, while the oil-loving part points toward the oil. This positioning reduces surface tension and keeps the mixture stable.

These emulsifiers were selected for their ability to improve the texture, spreadability, colour dispersion, and overall stability of the product. A high HLB value (between 10 and 18) indicates a more water-loving emulsifier, suitable for mixing oils into water. In contrast, a low HLB value (between 3 and 8) indicates a more oil-loving emulsifier, better for mixing water into oils (Al-Suwayeh *et al*., 2020). Among the four developed formulations, the strategic use of natural colorants combined with carefully chosen emulsifiers effectively addresses key formulation challenges. This approach not only enhances the performance of the product but also supports the development of safe, stable, and consumer-friendly herbal lipsticks. This study aimed to develop and evaluate herbal lipsticks using Beta vulgaris extract as a natural chromophore and diverse natural emulsifiers.

MATERIALS AND METHODS

Materials

This formulation is composed of premium ingredients sourced from trusted suppliers. Beeswax is acquired from Akhil Health Care Private Limited, while castor oil is obtained from Unilex Colours and Chemicals Limited. Coconut oil is sourced from Pranav Agro, and lecithin is supplied by Gayathri Global Ingredients. Lanolin is provided by Kuntal Organic LLP, whereas candelilla wax comes from Goldlan Pharmaceutical LLP. Fresh, natural components like beetroot and lemon juice are procured from the local market to ensure quality and purity. Finally, rose essence from H R Aroma contributes a delightful fragrance to the formulation.

Methods

Method of preparation of herbal lipstick involve several steps like extraction of colour, adding of antioxidants and preservatives, dispersion of colour, melting of wax, mixing of wax, moulding and flaming as illustrated in Figures 1, 2A and 2B (Kolekar and Hingane, 2022).

- **Extraction of colour:** Peeled beetroot were cut into small dices and the pigment was extracted by boiling the pieces in distilled water for 9-11 min. The extract was then filtered. The filtrate was subsequently reboiled to concentrate the solution and obtain a thicker consistency.
- **Adding of antioxidant and preservative:** In the next step, an antioxidant was added into the aqueous phase.
- **Dispersion of colour:** Once the colour extract was prepared, it was heated in a water bath alongside the oil mixture in separate beakers. The emulsifying agent was added to the oil phase. When both phases reached a temperature of 70°C, the aqueous phase was gradually added to the oil phase with continuous stirring to ensure proper emulsification.
- **Melting of wax:** At the next, the wax was melted under controlled heating.
- **Mixing of wax:** Once the wax was fully melted, it was slowly incorporated into the colour mixture with gentle stirring to prevent air entrapment. Subsequently, the flavoring agent was added.
- **Moulding:** The prepared mixture was then carefully poured into a mold, leaving a small space at the top. The mold was left undisturbed at room temperature for 20 min to allow the lipstick to solidify
- **Flaming:** Finally, the lipstick sticks were inserted into containers, and reheating was performed on the exposed ends to achieve a smooth finish (Sharma, 2008). By following the above process, one batch of lipstick was prepared for each formulation with a total batch size of 50 g, based on the composition provided in Table 1 for a single 4.5 g lipstick unit.

Evaluation tests

Evaluation is the tool by which the quality of an formulation can be determine. For herbal lipstick these are melting point, breaking point, force of application, surface anomalies etc.

- **Solubility test:** Initially, 5 mL of each solvent was poured into separate test tubes. The lipstick sample was then added to each tube, followed by continuous stirring to facilitate dissolution.
- **Melting point test:** The determination of the melting point is crucial as it indicates the safe storage limit of the product. In the case of the formulated lipstick, the melting point can be measured using the DSC.
- **pH test:** For the pH measurement determination pH meter method was adopted. Initially the pH meter was calibrated using standard buffer solutions. After

calibration, the electrode was immersed in the lipstick dispersion, and the pH value was recorded. This procedure was repeated three times, and the average pH was calculated (Dhadwal *et al*., 2023).

- **Colour intensity test:** A spectroscopic method was employed to determine the colour intensity. Initially, 10 mg of each lipstick sample was dissolved in 70% ethanol to obtain a clear solution. The undissolved particles were then removed through filtration, followed by UV-visible spectroscopy analysis. Finally, the absorbance values were compared to assess the colour intensity.
- **Breaking point test:** The breaking point was used to determine the strength of the lipstick. The lipstick was positioned horizontally in a socket, placed half (1/2) inch from the edge of the support. The weight was gradually increased by a specific amount at 30 sec intervals. The weight at which the lipstick broke was recorded as its breaking point (Hingane *et al*., 2024).
- **Surface anomalies test:** The Surface Anomalies Test for lipstick is performed to assess the surface quality of the product, ensuring it is smooth, consistent, and free from visible imperfections. The lipsticks were undergoes visual examination under appropriate lighting to identify any defects such as cracks, air bubbles, oil sweating, uneven texture or colour (Mishra and Dwivedi, 2012).
- **Transparency test:** It is an in-house method conducted using tissue paper. Initially, lipstick marks were made on a tissue paper. Another tissue paper was then placed over the marked area, followed by the application of pressure to assess the transferability of the lipstick (Pavithra *et al*., 2022).
- **Water resistance test:** It had involved applying lipsticks to the skin, followed by exposing the area to running tap water to evaluate its resistance to water.
- **Perfume stability test:** The perfume stability test for lipstick is performed to assess the durability and consistency of the fragrance over time. The test is carried out in three steps. First one was initial assessment, where the fragrance of the lipstick were evaluated immediately after preparation. Next one is storage conditions, where samples were stored under different conditions. Like that third one is periodic evaluation where the lipsticks were examined at each two-month intervals over a period of six months.
- **Skin irritation test:** The skin irritation test for lipstick is performed to assess the product's safety and confirm it does not trigger adverse skin reactions. A small quantity of lipstick was applied to a clean, dry area of skin, such as the inner forearm of volunteers. The applied area was then left undisturbed for 24 to 48 h. This procedure was repeated for each formulation (Mishra and Dwivedi, 2012).
- **Aging stability test:** The aging stability test for lipstick is performed to assess the product's stability and performance over time, mimicking the effects of prolonged storage. Lipstick samples were kept in their packaging and stored under various conditions, including room temperature and elevated temperatures (40ºC-45ºC) for a duration of one month (Kolekar and Hingane, 2022).
- **Thixotropic behaviour test:** To assess the thixotropic behaviour of a lipstick formulation, ensuring smooth application and structural stability, the lipstick samples were gently melted and tested using a rheometer to determine their initial viscosity. The samples were then exposed to continuous shear stress through high-speed stirring or rotational force for 5 min. Once the shear force was removed, the samples were left undisturbed for 30 min to allow them to recover their original structure. Viscosity measurements were taken at regular intervals during the recovery period (Mishra and Dwivedi, 2012).
- **Antimicrobial test:** The antimicrobial test for lipstick is conducted to evaluate the product's ability to resist microbial contamination and ensure its safety for consumer use. The inoculated samples were incubated under controlled conditions 30-35ºC and 20-25ºC for a specific 7 days.

Phytochemical screening

Phytochemical screening of Beta vulgaris water extract was performed to detect the presence of different bioactive compounds, including alkaloids, flavonoids, saponins, tannins, and phenolic compounds.

Differential scanning Calorimetry

A DSC-6100 (Seiko Instruments) equipped with a thermal analyzer was used for the analysis. Precisely weighed samples (approximately 2 mg) were placed in sealed aluminum pans and heated under a nitrogen flow of 20 mL/min. The heating was carried out at a scanning rate of 10ºC per min, ranging from 30ºC to 150ºC (Priyadarshan *et al*., 2024).

RESULTS

The solubility test results for all the Formulations (F1-F4) indicate distinct solvent compatibility. All formulations showed partial solubility in distilled water, while they exhibited good solubility in 70% ethanol, acetone, and chloroform. Moderate solubility in isopropyl alcohol and poor solubility in hexane. Overall, the results showed the formulations are amphiphilic in nature, containing both polar and non-polar ingredients, results presented in Table 2.

Similarly, for the pH test, colour intensity test, breaking point test, surface anomaly evaluation, anti-transparency test, and water resistance test, different results were observed for different formulations. All formulations exhibited pH values within the acceptable dermal range (6.3-6.8), with F1 showing the highest (pH 6.8) and F3 the lowest (pH 6.3). In the colour intensity test, absorbance values ranged from 0.83 to 0.92, where F2 recorded the highest (0.92), indicating the most intense colour, and F1 the lowest (0.83). The breaking point test revealed that F1 had the highest mechanical strength (610 g), while F2 was the softest (450 g). All formulations showed no surface anomalies, reflecting smooth and uniform textures. Regarding anti-transparency test, F1 was rated very good. Similarly, for water resistance test, F1 demonstrated superior performance. The results are presented in Table 3.

The perfume stability test showed the fragrance retention for all the formulations. During the initial period of assessment, all the formulations were found to be stable, indicating no immediate loss or change in fragrance. However, upon periodic evaluation, a slight fade in fragrance was observed with all the formulations. The result are given in the Table 4.

In case of skin irritation test, formulations F2, F3, and F4 showed no indication of irritation, showing good dermal compatibility.

Table 1: Formulation of herbal lipstick.

Ingredient (For 4.5 g)	F1 (g)	F2 (g)	F3 (g)	F4 (g)
Bees wax	1.125	1.125	1.125	1.125
Castor oil	1.125	1.125	1.125	1.125
Coconut oil	0.562	0.562	0.562	0.562
Bees wax (E1)	0.562	--	--	--
Lecithin (E2)	--	0.562	--	--
Lanolin (E3)	--	--	0.562	--
Candelilla wax (E4)	--	--	--	0.562
Beet root	0.843	0.843	0.843	0.843
Lemon juice	0.140	0.140	0.140	0.140
Rose essence	0.140	0.140	0.140	0.140

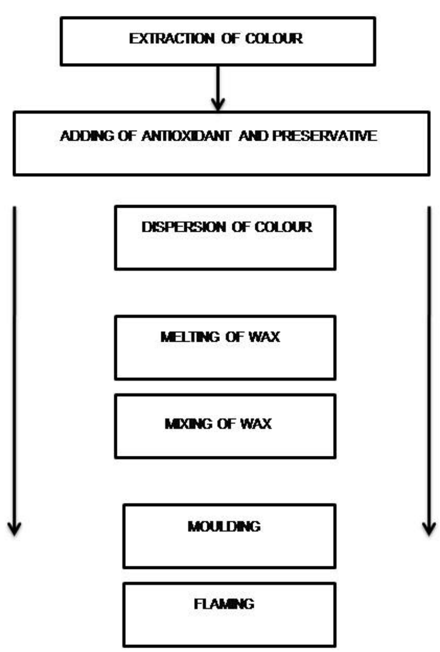

Figure 1: Schematic layout showing the steps of lipstick.

Table 2: Solubility test.

Solvent	F1	F2	F3	F4
Distilled Water	Partial	Partial	Partial	Partial
70% Ethanol	Good	Good	Good	Good
Isopropyl Alcohol	Moderate	Moderate	Moderate	Moderate
Acetone	Good	Good	Good	Good
Chloroform	Good	Good	Good	Good
Hexane	Poor	Poor	Poor	Poor

Table 3: pH test, Colour Intensity test, Breaking point test, Surface anomalies, Anti-transparency test and Water Resistance test.

Test	F1	F2	F3	F4
pH	6.8	6.5	6.3	6.7
Colour Intensity (Absorbance of F1 at λ_{max} 535 for 10ppm)	0.83	0.92	0.87	0.85
Breaking point	610g	450g	520g	560g
Surface anomalies	No Defect	No Defect	No Defect	No Defect
Anti-transparency	Comparatively Very good	Comparatively good	Comparatively good	Comparatively good
Water Resistance	Comparatively Very good	Comparatively good	Comparatively good	Comparatively good

Table 4: Perfume stability test.

Condition	F1	F2	F3	F4
Initial Assessment	Stable	Stable	Stable	Stable
Storage Conditions	Stable	Stable	Stable	Stable
Periodic Evaluation	Slight fade	Slight fade	Slight fade	Slight fade

F1 also did not cause irritation but was noted to cause slight discomfort. The results are given in the Table 5.

Initially, all formulations exhibited high viscosities in case of thixotropic behaviour test, with F1 and F4 at 150,000 cP, and F2 and F3 at 145,000 cP. After 10 min of shear, the viscosity of all formulations decreased significantly to 85,000-90,000 cP, indicating that the structure temporarily broke down under stress. Over time, the viscosity gradually recovered, reaching 110,000-120,000 cP after 20 min, and nearly returning to their initial values after 30 min. The result are given in the Table 6.

The antimicrobial test on all the formulations (F1-F4) under two different conditions to evaluate their ability to resist microbial contamination. As presented in Table 7, all formulations exhibited no growth.

Phytochemical screening of the formulations confirmed the presence of various bioactive compounds. As shown in Table 8, the tests showed positive results (+Ve) for several important phytochemicals.

The Differential Scanning Calorimetry (DSC) analysis showed the thermal behavior and stability of the Formulations (F1-F4). The melting endotherm observed for the formulations were 71.35°C for F1, 65.19°C for F2, 67.89°C for F3, and 68.96°C for F4, the same is given in Table 9 as displayed in Figure 3.

DISCUSSION

The comparative assessment for the Formulations (F1-F4) demonstrated that each exhibited unique physicochemical and functional properties influenced by their respective compositions.

In the pH evaluation test, all the formulations showed pH values within the dermally acceptable range for lip products i.e., from 5.5 to 6.8 (Wiley, 2023). This indicates a low potential for muco-cutaneous irritation and confirms their safety for topical application (Draelos, 2018). In the colour intensity test, F2 shows the highest absorbance, recorded as 0.92 at λ_{max} 535 nm. This result is attributed to the high concentration of betalain pigments present in the F2 formulation (Shrestha and Shrestha, 2024).

The mechanical strength analysis showed that F1 has the highest breaking point (610 g), indicating superior structural integrity. However, this increased hardness may compromise user comfort and ease of application. Formulations F2 with lower breaking strength, offer better application properties. Surface anomaly evaluation confirms that all formulations are free of cracks or deformities, reflecting uniform surface morphology. The transparency tests indicated a high retention time. F1 shows superior opacity, may be due to its higher wax content, which enhances pigment retention and reduces translucency.

Water resistance test shows that all the formulations withstand smudging and maintain their colour when exposed to moisture, which is a favorable trait for long-lasting lip products. The fragrance also remained stable throughout the study period, although a slight decline in fragrance is observed. The skin irritation tests confirmed dermatological safety, with no adverse reactions observed in volunteers except with mild discomfort in case of F1. This may be due to higher wax concentration, possibly affecting skin feel.

Stability is observed with all the formulations, with each maintaining its physical and organoleptic properties under standard storage conditions. This reflects long shelf-life performance and dependable product quality.

All the formulations exhibited thixotropic behavior of suitable rheological characteristics for cosmetic use. When subjected to shear stress, such as during application, their viscosity decreases significantly (from 145,000-150,000 cp to 85,000-90,000 cp), allowing for easy and smooth spreading (Mishra and Dwivedi, 2012). Notably, F1 and F4 fully recovered their original viscosity within 30 min, demonstrating efficient re-solidification, a key factor in preventing smudging after application.

The microbial limit test, performed according to USP, confirmed the microbiological safety of all the formulations, with no detectable bacterial or fungal growth (United States Pharmacopeia [USP], 2020). The phyto-chemical analysis identified the presence of flavonoids, phenolic compounds, and tannins bio-actives with known antioxidant, antimicrobial, and anti-inflammatory properties (Bainsal *et al*., 2021). These findings suggested added

Table 5: **Skin irritation test and Aging Stability test.**

Test	F1	F2	F3	F4
Skin irritation	No Irritation But slight discomfort	No Irritation	No Irritation	No Irritation
Aging Stability	Stable	Stable	Stable	Stable

Table 6: **Thixotropic behaviour test.**

Time	F1	F2	F3	F4
Initial (without Shear)	150,000cp	145,000cp	145,000cp	150,000cp
After 10min	90,000cp	85,000cp	85,000cp	90,000cp
After 20min	120,000cp	110,000cp	110,000cp	120,000cp
After 30min	145,000cp	140,000cp	140,000cp	145,000cp

Figure 2A: Preparation procedure.

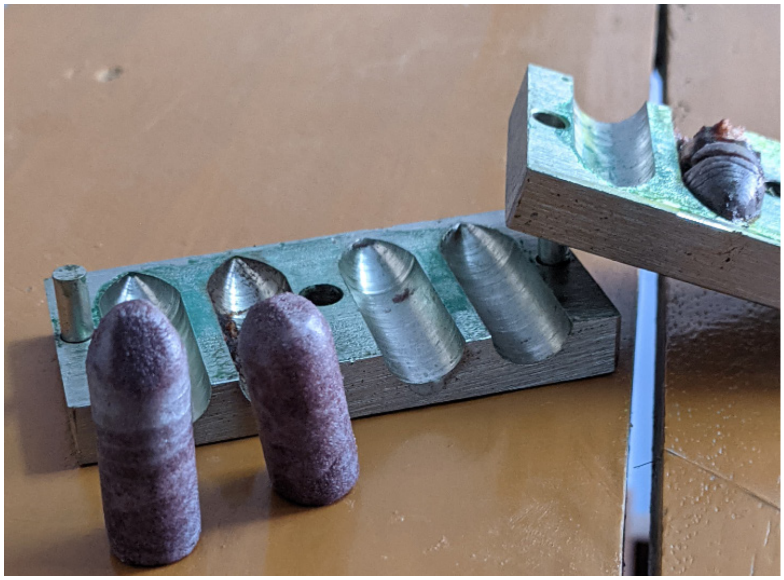

Figure 2B: Preparation procedure.

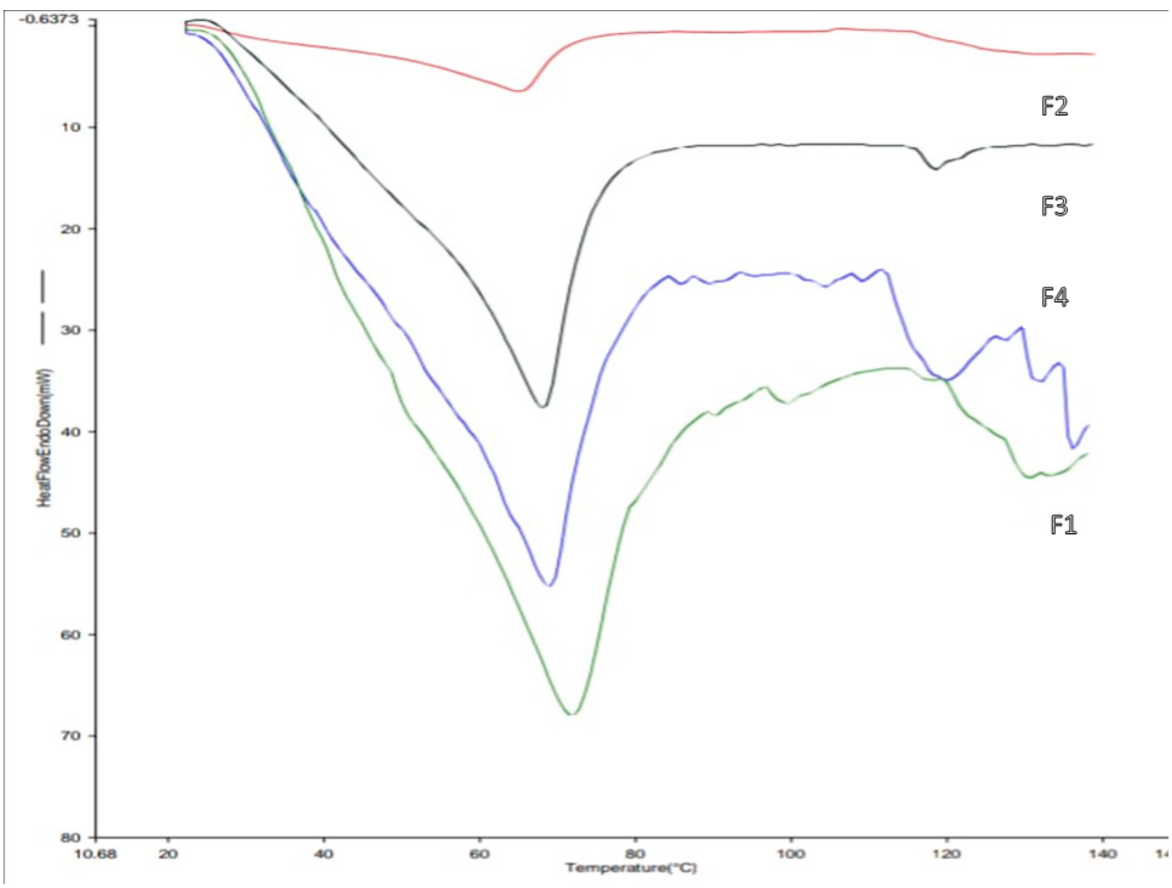

Figure 3: Thermograms of different lipstick Formulations (F1 to F4).

Table 7: **Antimicrobial test.**

Condition	F1	F2	F3	F4
Condition1	No growth	No growth	No growth	No growth
Condition2	No growth	No growth	No growth	No growth

Table 8: Phytochemical Screening.

Compounds	Test	Observation	Result
Alkaloids	Mayer's Test	Cream-coloured precipitate	+Ve
Flavonoids	Shinoda Test	Pink colour formation	+Ve
Saponins	Frothing Test	Froth formation	+Ve
Tannins	Ferric chloride Test	Blue colour	+Ve
Terpenoids	Salkowski Test	Red	+Ve
Phenols	Ferric chloride Test	Dark green	+Ve
Glycosides	Keller- Test	Reddish	-Ve
Carbohydrates	Molisch's Test	Purple ring	+Ve
Proteins	Biuret Test	Purple color	+Ve
Lipids	Spot Test	Oil spot	+Ve

Table 9: Differential scanning calorimetry.

F1	F2	F3	F4
71.35ºC	65.19ºC	67.89ºC	68.96ºC

therapeutic potential, extending the product's benefits beyond cosmetic use.

The DSC analysis provided the insight into thermal behavior. F1 exhibits the highest thermal peak (71.35ºC), confirming its excellent thermal stability. F2 has the lowest peak (65.19ºC), supporting its enhanced spreadability. F3 and F4 exhibit intermediate thermal events, suggesting a balance between application properties and heat resistance as displayed in Figure 3. All formulations demonstrated acceptable thermal profiles for commercial applications.

Collectively, these results highlight the versatility of natural emulsifiers and beetroot extract in developing herbal lipsticks with a favorable physicochemical, aesthetic, and safety attributes.

CONCLUSION

Based on the comprehensive evaluation of all the Formulations (F1-F4), each demonstrated acceptable performance with respect to physicochemical, microbiological, and stability evaluation parameters. However, certain formulations exhibited superior characteristics in specific tests. Among all the formulations, F1 caused slight discomfort in the skin irritation test, but exhibited the highest thermal peak (71.35ºC), confirming its excellent thermal stability. The formulation F2 had the most skin-friendly (pH 6.5) and showed no irritation, making it preferable for sensitive skin, while similarly, F2 exhibited the highest colour intensity (absorbance 0.92), suggesting enhanced pigment dispersion-likely due to the presence of an emulsifier with a low HLB value. It also showed the lowest breaking point (450 g), indicating a softer consistency. Furthermore, it demonstrated excellent thixotropic recovery, with viscosity effectively restoring over time, ensuring consistent spreadability and performance during application. The DSC data further support the good spreadability of the F2 formulation, with a peak temperature of 65.19ºC, indicating lower rigidity. All Formulations (F1-F4) passed the microbiological safety test, exhibited good perfume stability, had no surface anomalies, and remained within acceptable limits for other evaluation parameters, confirming their suitability for safe cosmetic use. Hence, each formulation had unique features. Considering all, in conclusion formulation F2 is emerged as the best formulation; offering high colour intensity, good spreadability, and satisfactory performance basing on evaluation criteria, making it the most promising herbal lipstick formulation among the four.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DSC: Differential Scanning Calorimetry; **FDA:** Food and Drug Administration; **HLB:** Hydrophilic Lipophilic Balance; **UV:** Ultraviolet.

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