

Herbal formulation and evaluation of cosmetics from *Bixa orellana* and *Beta vulgaris*

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Abstract

Bixa orellana L., commonly known as sindoori plant because of its coloring pigment in seeds belongs to the family Bixaceae. In the present study the coloring pigment of *Bixa* seeds with *Beta vulgaris* used for herbal lipstick formulation. The roots and seeds were collected, washed, dried and extract prepared in methanol. The extract percentage yield was found 38.3 % and 35.19 % in *B. orellana* and *B. vulgaris*, respectively. The qualitative analysis showed presence of quinones, flavonoids, cardiac glycosides, tannins, terpenoids, reducing sugar in both and phlobatannins, glycosides, steroids in *B. vulgaris*, whereas anthraquinones was also present in *B. orellana*. The thin layer chromatography using solvent chloroform and methanol (47:3) ratio gave presence of Bixin, Norbixin and Betalains on the basis of Retention factor. The herbal formulation includes natural coloring agent of both extracts 5 gm, with base of waxes include carnauba wax 5 gm as structural base, beeswax 5 gm as hardening agent, 1 mL of coconut oil as moisturizer, 1 mL glycerin humectant and rose water 2 mL as cooling and fragrance. The formulation was analyzed by evaluation parameters and results gave stable aging at varying temperature with melting point at 55 ° C, pH 6.8 ± 0.02, red color, smooth texture with good spreadability, breaking point 31.5, no surface anomaly and pleasant odor with no skin irritation, erythema or edema. The consistency was uniform not too hard or soft, chloroform solubility, force of application was good, grease test results showed matt lipstick texture. The results demonstrated stable, eco-friendly with no side effects of herbal formulation which can be used on daily basis by women with no side effects.

Keywords: *Bixa orellana*, *Beta vulgaris*, cosmetics, lipstick and phytoconstituents

Introduction

Cosmetics provide a sense of satisfaction and confidence in women and has become a regular and common practice especially lipstick. But regular use causes harm to skin due to presence of chemicals. Therefore, herbal formulations are becoming familiar and in demand (Varghese *et al.*, 2017)^[48]. The paradigm shift from synthetic to natural products has significantly influenced the global cosmetic industry. Consumers are increasingly prioritizing “clean beauty,” leading to a resurgence in the utilization of botanical extracts for personal care (Ahmed *et al.*, 2022)^[3]. Herbal cosmetics are defined as preparations containing phytochemical constituents derived from various plant parts to provide prophylactic or therapeutic benefits to the skin, hair, and nails. Unlike synthetic counterparts, which often contain parabens, phthalates, and lead-based pigments, herbal formulations offer a biocompatible profile, lower toxicity, and multifaceted biological activities including antioxidant, antimicrobial, and UV-protective properties (Bouissane *et al.*, 2025)^[13]. Herbal cosmetics products improve the functioning/texture of the skin by boosting collagen growth by eradicating harmful effects of free radicals, maintains keratin structure in good condition and making the skin healthier. There are numerous herbs available naturally having different uses in cosmetic preparations for skincare, hair care and as antioxidants (Aparna, 2017)^[7]. The most common and frequent cosmetic used by female is lipstick that is applied to the lips for color, texture and protection (Sunil *et al.*, 2013)^[42]. It generally contains oil, waxes, emollients and pigments. The pigments frequently used in lipsticks are synthetic chemicals that act

as a source of heavy metals. These heavy metals may be present in relatively small amounts, but their presence in the cosmetics formulation can induce hazardous conditions because the heavy metals are absorbed through the skin. It may also cause adverse effects such as allergy, dermatitis, skin discoloration and drying of lips (Azhar *et al.*, 2017)^[8]. The long-term usage for whole lifetime may be carcinogenic and fatal too (Varghese *et al.*, 2017)^[48]. This limitation thus leads the use of natural colourants as an alternative in lipstick. Lipsticks containing natural colourants are termed “Natural lipsticks” or “Herbal lipstick” as it derived from medicinal herbs and possesses pharmacological properties like antioxidant, antimicrobial, anti-inflammatory, and cytostatic effects. It is natural, safe, skin-friendly, non-toxic and has no side effects for humans (Swati *et al.*, 2013)^[43]. In the present study *Bixa orellana* and *Beta vulgaris* pigments are used for herbal lipstick formulation. *B. orellana* L. also known as sindoori plant belongs to the family of Bixaceae, possess medicinal and industrial properties (Vilar *et al.*, 2014)^[50]. The plant contains bright red seeds rich in natural pigments due to presence of bixin and norbixin, a carotenoid, responsible for characteristic reddish orange colour. These Pigments have been extensively used as natural colorants in food products, cosmetics, printing industries, dye manufacturing and textiles (Raju *et al.*, 2022)^[30]. In addition, most endogenous people use this dye on their skin to beautify themselves during religious rituals and mainly to protect themselves from ultraviolet radiation and from mosquitoes that infest forests (Barbosa-Filho *et al.*, 1998)^[10]. The other plant used in the present study was *Beta vulgaris* provides water-

soluble, intense but unstable pigments. When combined, they offer balanced pigmentation, improved antioxidant activity, better formulation versatility and enhanced aesthetic properties (Negi, 2025) ^[22]. *B. vulgaris* L. is a biennial herbaceous plant, taxonomically classified in the Amaranthaceae family. It contains bioactive nitrogen-based pigments betalains, water soluble with unique color varies from yellow to violet. These pigments serve as a natural food coloring agent (Babarykin *et al.*, 2019) ^[9] and possess pharmacological properties because of their valuable active components, betaxanthin, and betacyanin (Thiruvengadam *et al.*, 2022) ^[44].

The study aimed to formulate herbal lipstick composed of pigments, oils, waxes and emollients. These formulations are evaluated for various organoleptic characteristics, including melting point, Spreadability, hardness, skin compatibility, shine, and gloss, to ensure they offer a pleasing and attractive finish (Achal *et al.*, 2025) ^[1]. Primarily, the pH test was conducted to ensure the lipstick's compatibility with the natural pH of the skin, resulting in an optimal pH level that avoids skin irritation. The melting point test confirmed the product's stability across various temperature ranges, ensuring that it remains solid in typical environmental conditions. The force of application test measured the pressure required to apply the lipstick smoothly, indicating a user-friendly formulation. A skin irritation test was conducted with human volunteers, demonstrating that the lipstick does not cause adverse skin reactions.

Materials and Method

In the present study formulation and evaluation of an herbal lipstick prepared using natural colorants derived from *Bixa orellana* (Annatto) and *Beta vulgaris* (Beetroot). The study emphasizes the utilization of bioactive plant pigments to develop a safe, eco-friendly, and skin-compatible cosmetic alternative to synthetic formulations.

Materials and Reagents

The materials used in this study were selected based on their functional and physicochemical properties to ensure the development of a stable and safe cosmetic formulation free from harmful synthetic additives.

Active Ingredients (Colorants): Seeds of *Bixa orellana* (sindoor) and fresh roots of *Beta vulgaris* (Beetroot).

Excipients/Base Materials: Coconut oil (moisturizing agent/base), glycerin (humectant), carnauba wax, and beeswax or paraffin wax (hardening agent/binder).

Collection and preparation of plant extracts

The seeds *Bixa orellana* (Sindoor plant) were collected from herbal garden of Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal. The roots of *Beta vulgaris* (beetroot) were procured from local market. The seeds and roots were cleaned, washed, shade dried, powdered mechanically and stored in zip lock polythene bags for further experiment. The extract was prepared by soaking 10 g powder of plant part in 200 ml solvent (100 ml water + 100 ml methanol) for 24 h, filtered and air dried. The powder was further used for herbal formulation and other evaluation parameters.

Determination of Extraction Yield of Plant Extract (% yield)

The weight of dried plant part was calculated and its extract prepared and its percentage yield of the extract was calculated. The yield (% w/w) from all dried extracts was calculated by the formula given below.

$$\text{Yield (\%)} = \frac{W_2 - W_1}{W_0 \times 100}$$

Where, W_2 is the weight of the extract and the container, W_1 weight of the container alone and W_0 the weight of the plant powder (Anokwuru *et al.*, 2011) ^[6].

Phytochemical Analysis (Qualitative) of Plant Extract

The phytochemical analysis was carried out to determine the active ingredient of *Bixa orellana* and *Beta vulgaris* extracts, fraction of methanolic extract and fractionation residue of the plant was used for the detection of the presence of alkaloids, flavonoids, saponins, tannins and glycosides. Qualitative estimation of phytochemical analysis has been estimated on the basis of color formation (Tiwari *et al.*, 2011; Mir *et al.*, 2013; Trease and Evan, 1983; Kokate *et al.*, 1997; Hegde and Joshi, 2010) ^[16, 17, 20, 45, 46].

Anthraquinones (Borntrager's test)

The crude extract was weighed 0.5 g and boiled with 10% hydrochloric acid (HCl) for few minutes in a water bath, filtered and cooled. To the filtrate equal volume of chloroform (CHCl_3) and few drops of 10% ammonia was added and allowed to heat. The presence of anthraquinones were indicated by appearance of pink rose color.

Test for Quinones (Sodium Hydroxide (NaOH) Test)

Add 1 ml NaOH (10% in alcohol) 1 ml of plant extract. The development of a red, blue-green, or reddish-blue color indicates the presence of quinones.

Test for Glycosides

Filter plant material and add chloroform 3 mL mix and shake. Chloroform layer separated and to it 10 % Ammonia added. Appearance of pink color for positive result.

Test for Cardiac Glycosides (Keller-Kiliani Test)

Mix the chloroform extract with glacial acetic acid containing 2% (FeCl_3). Add concentrated H_2SO_4 to form a layer. A brown ring at the interface and a bluish-green turning layer indicate cardiac glycosides.

Steroids (Salkowski Test)

The crude extract was dissolved in methanol and to it 5 drops of concentrated Sulfuric acid (H_2SO_4) was added. The formation of red color indicated the presence of steroids.

Tannins (Ferric Chloride Test)

The crude extract was weighed to 0.5 g and dissolved in 10 ml of distilled water. The solution was filtered and to it ferric chloride was added, the presence of tannin was indicated by blue black precipitate.

Terpenoids (Salkowski Test)

The extract was weighed 0.2 g and mixed with 2 ml of chloroform and 3 ml of concentrated hydrochloric acid (HCl) carefully to form a layer. A reddish-brown coloration

of the interface formed indicated positive results for the presence of terpenoids.

Flavonoids

The crude extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min, filtered and mixed with 1 mL dilute ammonia solution to 4 mL filtrate. The formation of yellow colour indicates the presence of flavonoids.

Reducing Sugar

The crude extract was weighed 0.5 g in test tube and dissolved in 1 ml of distilled water and to it 2 to 8 drops of Fehling solution was added and boiled for few minutes. The brick red precipitate is formed indicated the presence of reducing sugar.

Phlobatannins

The extract of plant sample was boiled with 1% aqueous hydrochloric acid (HCl) to observe the deposition of red precipitate for the presence of phlobatannins.

Thin Layer Chromatography (TLC) Analysis

Thin Layer Chromatography (TLC) was carried out for the preliminary identification and separation of phytoconstituents present in the extracts of *Bixa orellana* and *Beta vulgaris*. The analysis was performed to confirm the presence of pigment-containing compounds and evaluate the chemical profile of the extracts. Silica gel powder was taken and dissolved in distilled water mixed using mortar and pestle. The prepared gel poured on clean slides and slides kept for drying an activation for one hour at 110° C. Ten (10 µL) of 5% solution of the extract in 95% methanol was spotted on the plate and 10 µL was added to the plate. The plate was allowed to dry and develop in a mixture of chloroform and methanol (47:3 by volume) until the solvent front had ascended to 10 cm. The slides were then removed, dried in oven and the height of the solvent was marked using a pencil. The slides were developed using iodine crystals. It was allowed to dry and the retention factors of the color spots were determined. The Retention factor (Rf) values for each spot was calculated. The Rf is defined as the distance traveled by the compound divided by the distance traveled by the solvent.

$$\text{Rf value} = \frac{\text{distance travelled by compound}}{\text{distance travelled by solvent}}$$

Formulation of Herbal Lipstick

The herbal lipstick was prepared by using extracts of *Bixa orellana* and *Beta vulgaris*, and other ingredients in which the ratio of ingredients was optimized to achieve desirable color intensity, consistency, and structural stability. The carnauba wax 5 gm and beeswax 5 gm was melted at 70 ° C using water bath. The beeswax acts as hardening agent. Allow the temperature to reduce to 50 ° C and to it 1 mL of coconut oil containing 5 gm extract of each *B. orellana* and *B. vulgaris* added to the molten wax with continuous stirring. The extract of *Bixa orellana* act as natural pigment and *Beta vulgaris* as natural pigment and antioxidant. After addition of extracts, glycerin 1 mL added which acts as softener or humectant. To the mixture two capsules of vitamin E was also mixed which acts as antioxidant. The formulation was stirred thoroughly to ensure uniform dispersion of all components and to eliminate air bubbles.

Finally, for fragrance rose water 2 mL was added, and the molten mixture was poured into pre-lubricated lipstick molds. The molds were then refrigerated for 15–20 minutes to allow complete solidification.

Evaluation Parameters

The formulated herbal lipstick was evaluated to assess its quality and effectiveness. To ensure its safety, stability, and usability.

Organoleptic Evaluation

The formulation was assessed visually for color, odor, texture, and consistency (not too runny or hard).

pH Determination

The pH was measured using a calibrated digital pH meter and pH strip to ensure compatibility with human skin (target range: 5.5–6.5). Dissolve 1 gm of test sample in a 10 ml of distilled water. Determine the pH of the test sample by using pH strip (Pratiksha *et al.*, 2024) [26].

Melting Point

The melting point was determined using the capillary tube method to evaluate thermal stability. The desired range was 55–65°C, ensuring stability at room temperature while allowing smooth application (Sandhyarani and Utkarsha, 2023) [35].

Solubility Test

The solubility of the formulation was tested in different solvents, including water and alcohol, to assess stability and resistance to dissolution (Venkata and Prasanna, 2024) [49].

Breaking point

The breaking point method was used to determine herbal lipstick strength. The lipstick was held horizontally in a socket, one inch from the support's edge. The breaking point was defined as the weight at which it broke, and it was increased gradually by a specified quantity (10 gms) at predefined intervals of 30 seconds (Achal *et al.*, 2025) [1].

Test for spreadability

Spreadability was tested using glass slides. The formulation was placed between two slides, and a load was applied to spread the formulation over the slides. Visual evaluation was conducted to assess the uniformity of the layer formed (Alessandrea and Michelli, 2013) [4]. For this test, the following criteria were established:

G – Good: uniform

I – Intermediate: uniform with few discontinuities

B – Bad: not uniform

Force of application

It is test for comparative measurement of the force to be applied for application. A piece of coarse brown paper kept on a shadow graph balance and lipstick was applied at 45° angle to cover a 1 sq. inch area until fully covered. The pressure reading is an indication of force of application (Nuha *et al.*, 2020) [23].

Grease test

Greasiness test was examined to identify the amount of oil in the formulation. Lipstick was placed on the filter paper and the sample was left at room temperature for 24 hours.

The diameter of produced oily ring was measured (Sherin *et al.*, 2023) [38].

Surface anomalies

The presence of surface anomalies, such as crystals or contamination by molds and fungi, can affect the overall quality and appearance of the lipstick. Formation of wrinkles, exudation of liquid substances and of solid fatty substances were also determined (Vrushali and Sakshi, 2025) [52].

Aging Stability test

The herbal lipstick formulation was tested for stability over a period of 30 days under different temperature conditions, including room temperature ($25 \pm 2^\circ\text{C}$), higher temperature in an oven ($40 \pm 2^\circ\text{C}$), and refrigerator ($5 \pm 2^\circ\text{C}$). The organoleptic properties and spreadability were assessed on the 7th, 15th, and 30th day. Perfume stability after 30 days, the prepared herbal formulation was evaluated for smell after being kept in a typical cold storage environment (Von *et al.*, 1972) [51].

Skin Sensitivity

It was carried out by applying the product in the form of a patch on the skin for 10 min, 30 min and 24 hours on healthy individual to check skin irritation, observe the reaction as signs of erythema, edema or irritation observed in healthy individuals (Sherin *et al.*, 2023) [38].

Statistical analysis

All experiments were performed in triplicate and results are expressed as mean $\pm$ standard deviation (SD). The mean, standard deviation and percentage reduction values were calculated in MS excel.

Results and Discussion

The prepared extract of *Bixa orellana* seeds and *Beta vulgaris* roots gave percentage yield 38.3 % and 35.19 % respectively, as maximum yield obtained due to solvent methanol. Methanol is a polar solvent and maximum phytochemicals were obtained in it. The crude extract separation is influenced by number of factors such as method of extraction, solvent type used, solvent amount, time and temperature, target component selection, amount of sample, and extraction efficiency. The nature of the solvents is the most crucial of these to dictates the target compound's solubility and extraction efficiency. Depending on the kind of extraction, as well as the solvent and temperature utilized, water-soluble colorants (norbixin) and oil-soluble colorants (bixin) can be obtained (Smith, 2006) [40]. *B. vulgaris* roots (BvRM) extract with a good percentage yield was obtained in methanol solvent with 54.06 % (Sidra *et al.*, 2021) [39]. Extraction with oil or solvent primarily yields a colorant called bixin (Mortenson *et al.*, 2008) [21]. Different studies used various organic solvents such as ethanol, chloroform, chlorinated hydrocarbons, acetone, ethyl acetate, hexane, methanol, or alcoholic sodium hydroxide, etc., either alone or in combinations at different concentrations or ratios for efficient extraction (Ramamurthy and Krishna, 1982) [31].

Phytochemical Analysis

The phytochemical analysis involves qualitative analysis of *B. orellana* seeds and *B. vulgaris* roots. The preliminary

qualitative tests have been attempted in crude extracts of to find out the presence or absence of certain bioactive compounds. The phytoconstituents obtained in both extracts include quinones, flavonoids, cardiac glycosides, tannins, terpenoids and reducing sugar. The *B. orellana* also gave presence of anthraquinones, whereas, *B. vulgaris* gave phlobatannins, glycosides and steroids. The studies done previously showed methanolic leaf extract of *B. orellana* was found to be rich in alkaloids, tannins, proteins, terpenoids, flavonoids, and steroids (Raymond *et al.*, 2023) [32]. The aqueous, alcoholic and hydro-alcoholic extracts of seeds and leaves showed the presence of various phytoconstituents i.e. carbohydrates, proteins, amino acids, steroids, glycosides, saponins, flavonoids, alkaloids, tannins and phenols (Sangeeta *et al.*, 2015; Radhika *et al.*, 2010) [27, 36]. The presence of secondary metabolites as phytoconstituents are responsible for its medicinal properties like anti-bacterial, anti-hyperglycaemic, anti-histaminic, anti-diarrheal, anti-cancer, anti-inflammatory and anti-oxidant activities (Umesh *et al.*, 2016) [47]. The phytochemical analysis of leaves and roots extracts of *Beta vulgaris* commercially grown in Punjab and Khyber Pakhtunkhwa (KPK) regions in Baluchistan and Sindh regions of Pakistan, showed presence of flavonoids, phenols, alkaloids, saponins and glycosides. The phytoconstituents are responsible for its antioxidant, anti-inflammatory and cholinesterase inhibitors as well as help in preventing and treating neurogenerative disorders (Sidra *et al.*, 2021) [39].

Thin Layer Chromatography

The results of thin layer chromatography on the basis of Rf value were calculated and found out that the seeds of *B. orellana* contains Bixin, a carotenoid and Norbixin with Rf value 0.23 and 0.61 respectively. The results of *B. vulgaris* of TLC on the basis of Rf value was calculated and may showed presence of Betalain and β – carotenoid with Rf value 0.40 and 0.61 respectively. The previous studies showed that seeds of *B. orellana* ethanolic extract gave Rf value of 0.56 and 0.42 for Bixin and Norbixin, respectively (Aluko, 2024) [5]. A number of studies reported separation of compounds Bixin (9Z-6, 6'-diapocarotene-6, 6'-dioate), and Norbixin, carotenoid-based coloured compounds separated from seeds of the tropical tree *B. orellana* L used primarily as a natural colouring agent in the food and cosmetic industry (Deborah *et al.*, 2025) [14]. In another study done in Chattisgarh and Uttaranchal, India, seeds extract characterized by TLC showed presence of bixin as major component in highest amount (Pattanaik *et al.*, 2018) [25]. Similarly, major compound of *B. vulgaris* is Betalains separated by thin layer chromatography on the basis of polarity and Rf value by chloroform extraction (Yalaka *et al.*, 2023) [53]. Betalains are water-soluble pigments and the most common betacyanin is betanin (Beetroot Red), present in red beets *Beta vulgaris*, which is a glucoside of betanidin. It has antioxidant property and used as a coloring agent in food and cosmetics (Sadowska-Bartos, and Bartosz, 2021) [33].

Herbal Formulation of lipstick

The herbal lipstick was prepared by using extracts of *Bixa orellana* and *Beta vulgaris*, and other ingredients in which the ratio of ingredients was optimized to achieve desirable color intensity, consistency, and structural stability. The

ingredients used and its function are given in tabular form (Table 1). The ingredient Beewax and carnauba wax are used as a thickening agent, helps to retain to moisturizing skin. It is used in number of cosmetics especially in lip – balm, lip gloss and hand creams (Meher *et al.*, 2011; Bharat *et al.*, 2011; Sharma, 2005) ^[11, 18, 37]. Beeswax is produced from the honeycombs of *Apis mellifera* and other species of the *Apis* genus, which belong to the Apidae family. Commonly used in preparing ointments, plasters, and various types of polishes. Widely utilized in the cosmetic industry for making products like face creams and lipsticks (Achal *et al.*, 2025) ^[1]. Carnauba is a plant-derived exudate obtained from the leaves of the Brazilian palm tree. Chemically, it is composed of fatty acid esters, hydroxylated fatty acids, and cinnamic acid derivatives. It possesses the highest melting point among natural waxes (82 to 86 °C), which is vital for the lipstick’s structural integrity, by acting as the primary “hardening agent,” it provides the necessary tensile strength to prevent the stick from snapping during application. Furthermore, its high refractive index contributes to a natural, high-gloss finish without the need for synthetic luster agents like polybutene (Raja, 2022) ^[28].

Another ingredient coconut oil is extracted from the dried endosperm (kernel) of the coconut fruit, obtained from *Cocos nucifera* L., a plant belonging to the Palmar (Arecaceae) family. It is applied topically, helps to moisturize and nourish the skin. It is also commonly incorporated into lipstick formulations for its hydrating properties. Addition of coconut oil acts as softener, moisturizes skin and protect from UV rays. Two herbal extracts used *B. vulgaris* and *B. orellana* seeds. Both act as a natural coloring agent, helps to provide a glossy and attractive shine to the lips (Achal *et al.*, 2025) ^[1]. The *B. vulgaris* extract acts as natural moisturizer for lips, helps in getting rid of darker lips and makes lips brighter and lighter (Sukanya, 2025) ^[41]. In the formulation vitamin E two capsules were added which act as a powerful antioxidant, protects body cells from damage caused by harmful free radicals. (Achal *et al.*, 2025) ^[1]. Its topical use relieves chapped, dry lips and prevent irritation. Glycerine moisturizer skin, gives glossy texture and enhance colorants (Aditi *et al.*, 2025) ^[2]. Rose water gives fragrance, act as soothing agent, maintain pH balance of skin and hydrate skin (Pathan *et al.*, 2025) ^[24].



Fig: Herbal lipstick formulation using *Bixa orellana* and *Beta vulgaris*

Table 1: Herbal formulation of lipstick

S.No.	Ingredient	Quantity	Function
1.	Beewax	5 gm	Structural base
2.	Carnauba wax	5 gm	Hardening agent
3.	Coconut oil	1 mL	Moisturizer
4.	<i>B. vulgaris</i> extract	5 gm	Natural pigment
5.	<i>B. orellana</i> extract	5 gm	Natural pigment
6.	Vitamin E	2 capsules	Antioxidant
7.	Glycerin	1mL	Humectant
8.	Rose water	2 mL	Cooling and fragrance

Evaluation Parameters

The formulation was evaluated to assess its quality and effectiveness to ensure its safety, stability and usability (Table 2). The pH determined to check potential negative effects. The pH was maintained neutral as acidic or alkaline may cause irritation to lips as pH of lips is neutral (Pratiksha *et al.*, 2024) ^[26]. The lipstick was found stable at room temperature by determining melting point. The solubility tests using different showed solubility in chloroform and insoluble in rest solvent to check its longevity (Table 3). The breaking point determine its strength was found 31.5 gm. As demonstrated by other studies formulations made with cold pressed coconut oil exhibited breaking point of 36.4 gm and formulations made up of cow ghee, cow ghee with coconut oil and only beewax exhibited 32 – 37 gm, 55 – 58 gm and 60.9 to 61.9 gm respectively (Venkata *et al.*,

2024) ^[49]. The absence of coconut oil and ghee has decreased its breaking point but softness also reduces. In the present study coconut oil was used and therefore, breaking point is acceptable. The uniform layer formation was determined by spreadability test and force of application was found smooth with ease and good. The formulated lipstick showed no surface anomalies with no crystals or contamination, no wrinkles formation, no liquid substance and no fatty substances.

The greasiness test results showed 4 cm diameter after 24 hours. The diameter size indicated that the lipstick was found to be firmer, drier, and matt. This offers high adherence, minimal smudging, and a lower grease feel (Esposito and Kirilov, 2021) ^[15]. The diameter indicates oil content and glossiness of herbal lipstick small diameter ($\leq 3 - 4$ cm) offers high adherence and low grease with matt lipstick texture. Large diameter ring (5 – 8 cm) indicates high oil content, glossy and spreadable on lips but have a higher chance of bleeding or smudging (Sherin *et al.*, 2023) ^[38]. The diameter 6 – 8 indicates moderate spread to ensure smooth glide and lip hydration without feeling overwhelmingly glossy (Miksusanti *et al.*, 2025) ^[19]. The aging stability at different temperature for 30 days was found stable, but high temperature $40 \pm 2^\circ\text{C}$, lipstick was kept in oven and was found unstable till 20 days and started releasing oil, but smell still remain pleasant, and texture was smooth.

Table 2: Evaluation parameters to check efficacy of herbal lipstick

S. No.	Evaluation Parameter	Observation
1.	Color	Red
2.	Odor	Pleasant
3.	Texture	Smooth and matt
4.	Consistency	uniform
5.	Homogeneity	viscous
6.	pH	6.8 ± 0.02
7.	Melting point	55 ° C
8.	Aging stability	Smooth
9.	Surface anomalies	No defect
10.	Solubility test	Chloroform
11.	Skin irritation test	No
12.	Perfume stability	Good
13.	Breaking point	31.5
14.	Force of application	Good
15.	Spreadability	Smooth
16.	Grease test	4 cm diameter

Skin Sensitivity

The 20 healthy volunteers were selected include students and staff of Paramedical Institute, Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal. The herbal formulation lipstick was applied in the form of patch on the skin for 10 min, 30 min and 24 hours. No redness was seen, no reaction, itching, swelling or inflammation was seen on the volunteer hands after 24 hours. No sign of erythema or edema was observed and formulation was found safe for use.

Table 3: Solubility test of herbal lipstick

S. No.	Solvent	Solubility
1.	Water	Insoluble
2.	Methanol	Insoluble
3.	Ethanol	Insoluble
4.	Isopropyl Alcohol	Insoluble
5.	Chloroform	Soluble
6.	Petroleum ether	Insoluble

In another formulation of herbal lipstick using natural sources such as carrot, beetroot, turmeric, tomatoes, pomegranate, and cocoa. The other different natural ingredients such as beeswax, carnauba wax, vanilla essence, castor oil, white soft paraffin, strawberry essence, vitamin E, lemon juice was used to formulate herbal lipstick. Prepared herbal lipstick was evaluated for different evaluation tests such as color, texture, pH, melting point, breaking point, surface anomalies, aging, and perfume stability (Nuha *et al.*, 2020) ^[23]. A trial was done to formulate natural lipsticks by exploitation colouring pigments of *Bixa orellana* linn seeds and *Beta vulgaris* linn root, Olive oil, ready organic product powder of shikakai on account of various antagonistic impacts of open manufactured readiness (Sainath *et al.*, 2016) ^[34]. Similarly, herbal lipstick formulation composed of shea butter, carnauba wax, castor oil, and natural aromatic essences of vanilla and rose was prepared and was optimized by varying wax-to-oil ratios to achieve desirable firmness, spreadability, stability, and sensory appeal by molding method. Carnauba wax served as the primary structuring agent, shea butter provided emollient and anti-inflammatory benefits, and castor oil acted as the moisturizing continuous phase. Evaluation studies showed a smooth texture, pleasant fragrance, absence of surface defects, pH (6.4 ± 0.2) and a

melting point range of 62–68°C, indicating good thermal stability. Mechanical strength, spreadability, and in-vivo skin irritation tests confirmed adequate durability, uniform application, and excellent biocompatibility (Bhavesh *et al.*, 2026) ^[12]. A number of lipstick formulations used beetroot as natural pigment (Sandhyarani and Utkarsha, 2023; Vrushali and Sakshi, 2025) ^[35, 52] and antioxidant with beeswax, cocoa butter, castor oil, and essential oils. The formulation exhibited excellent consistency, smooth texture, pleasant odour, and a natural red tint. The pH value remained within the dermal safety range (5.5–6.5), and the product demonstrated good physical stability without signs of phase separation or microbial contamination under varying storage conditions (Aditi *et al.*, 2025) ^[2]. Other studies of herbal lipstick formulation in which natural coloring agent *Bixa orellana* (annatto) and *Beta vulgaris* (beetroot) was used like present study. The formulation process involved waxes, oils, and pigments to achieve the desired texture, color, and stability. Primarily, the pH test was conducted to ensure the lipstick's compatibility with the natural pH of the skin. All tests yielded results within the acceptable reference ranges, validating the formulation's success (Rajeswari *et al.*, 2024) ^[29].

Conclusion

The present study showed that seeds of *Bixa orellana* and *Beta vulgaris* as a natural ingredient with base of waxes containing vitamin E and coconut oil was used for herbal formulation of lipstick. The plant extracts were prepared and analysed its constituents by phytochemical analysis and thin layer chromatography. The results showed presence of Bixin, Norbixin and Betalins. These constituents are responsible for pigment production in both the plants. These pigments give a natural coloring agent. The formulation was analysed by evaluation parameters and results gave stable aging at varying temperature with melting point at 55 ° C, pH 6.8 ± 0.02, red color, smooth texture, breaking point 31.5 and pleasant odour with no skin irritation. The results demonstrated stable, eco-friendly with no side effects of herbal formulation which can be used on daily basis by women with no side effects.

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