



Evaluation Of Beetroot Extract Lip Balm Antioxidant Study And Detection Of Methyl Paraben By Using Uv-Visible Spectroscopy And Hplc

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Abstract: This study investigates the antioxidant potential of a beetroot-based lip balm using the DPPH assay and assesses its safety by detecting methyl paraben through UV-Visible spectroscopy and HPLC. Beetroot is rich in antioxidants, including betalains and phenolic compounds. The DPPH free radical scavenging assay was used to evaluate its antioxidant activity. Furthermore, UV-Visible spectroscopy and HPLC were utilized to detect the presence of methyl paraben, ensuring adherence to safety regulations. The results emphasize beetroot's potential as a natural antioxidant in cosmetic applications. This research supports the development of herbal lip care products with enhanced functionality and reduced synthetic additives.

Keywords: Beetroot, Antioxidant, DPPH assay, Free radical scavenging, UV-Visible spectroscopy, HPLC, Methyl paraben

1. INTRODUCTION

Cosmetics are an essential part of modern life, and with the growing trend of adopting eco-friendly practices, many industries, including cosmetics, are embracing a more natural approach. There is a strong demand for organic plant-based products, and the use of herbal cosmetics in personal care has increased. Natural ingredients have been used in traditional medicine worldwide for centuries, with many providing medicinal properties such as antibacterial, anti-inflammatory and cytostatic effects. These natural remedies are widely acknowledged for their benefits in human healthcare. Cosmetics derived from plant extracts, especially for skin and hair care, are favored for their reliability. Herbal cosmetics contain valuable bioactive compounds that enhance skin health by delivering essential nutrients. Herbal remedies from traditional cultures are gaining recognition as natural alternatives to synthetic chemicals. Lip balms are designed to hydrate and

protect the lips from dryness and external factors. Since the skin on the lips is thinner, lacks hair follicles, sebaceous glands, and sweat glands, and is prone to moisture loss, it requires special care and protection.^[1] Many ingredients are included in skin care products, and they should be non-toxic, harmless, and safe to use. Various ingredients are used in cosmetic formulations, with polymers playing a key role. The most commonly produced cosmetic products typically contain ingredients such as water, emollients, antioxidants, UV filters, preservatives, humectants, and surfactants. Parabens are often used as preservatives in these formulations.^[2]

2. LIP BALM

Lip balm is a soft, waxy product used to moisturize and soothe dry or chapped lips. It was first introduced in the 1880s by Charles Browne Fleet. The skin on the lips is thin, making it more susceptible to dryness compared to other areas of the skin. Lip balm helps protect the lips from harsh conditions like dry air, wind, and cold temperatures. The ingredients in lip balm help keep lips hydrated and support the healing of chapped skin. Unlike lipstick, lip balm is not intended for a specific gender, and most formulas have a waxy texture. Some lip balms also contain medicated ingredients that promote healthy lips. Regular use of lip balm can prevent chapped lips, and applying it before exposure to sun, wind, or dry conditions provides extra protection.^[3]

2.1. TYPES OF LIP BALM

There are 6 types of lip balms: ^[4]

- **Tinted Lip Balm:** Tinted lip balm not only moisturizes the lips but also adds a light wash of color. It's a great option for those who want hydration and a subtle tint without the full coverage of lipstick.
- **Medicated Lip Balm:** Medicated lip balm is a specialized lip care product often recommended by doctors for treating chapped lips and other lip conditions. They typically contain active ingredients, such as hydrocortisone, that help treat issues like bacterial infections.
- **Flavored Lip Balm:** Flavored lip balm infused with various flavors like vanilla, mint, or fruit scents. It moisturizes the lips while providing a delightful taste and fragrance, making it a fun and refreshing experience.
- **Organic Lip Balm:** Organic lip balm is made from natural or organic ingredients, offering a healthier alternative to those that contain synthetic chemicals. It usually contains nourishing ingredients like avocado oil, jojoba oil, beeswax, vitamin E, hemp, and cocoa butter, which are gentle on the lips and skin.
- **SPF Lip Balm:** SPF lip balm contains ingredients that protect the lips from harmful UV rays, acting like sunscreen. It prevents sunburn and reduces the risk of lip damage or skin cancer, making it perfect for outdoor activities.
- **Plumping Lip Balm:** Plumping lip balm not only hydrates the lips but also enhances their appearance by making them look fuller and more defined. Special ingredients in these balms give the lips a more voluminous, plump look while also providing protection.

3. ANATOMY OF LIP

The lips are made up of the skin, superficial fascia, orbicularis muscle, and the surrounding muscles. The edges of the lips are covered with a dry, red mucous membrane, which is continuous with the skin and contains many small blood vessels and touch receptors. Internally, the mucous membrane is reflected from the upper and lower lips onto the gums, and in the center, it forms two folds: the superioris and inferioris. The lips serve several functions, including speaking, suction, and grazing. The mucous membrane at the lip borders is attached to the coronary vessels, which completely encircle the opening of the mouth near the lips' free edge, located in the areolar tissue or submucous layer^[5]

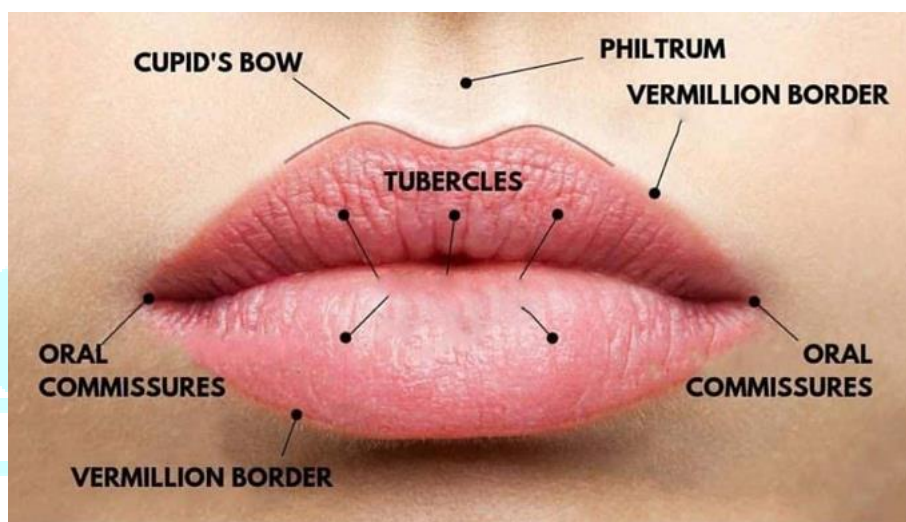


Fig 1: ANATOMY OF LIP

4. BEETROOT AS MAIN INGREDIENT

The beetroot, or beet, is the taproot of the *Beta vulgaris* subsp. *vulgaris* plant from the Conditiva Group. This root vegetable is commonly known as the table beet, garden beet, or dinner beet, and is often categorized by its color, such as red beet or golden beet. The plant also produces beet greens, which are the edible leaves. Beetroot can be consumed raw, roasted, steamed, or boiled, and can also be canned, either whole or chopped. It is frequently pickled, spiced, or served in a sweet-and-sour sauce. Beetroot is one of the cultivated varieties of *Beta vulgaris* subsp. *vulgaris*, grown for its edible roots and leaves, and belongs to the Conditiva Group. Beetroot is rich in antioxidants like vitamin C and betaines, which help protect the lips from damage and keep them looking healthier and younger. Beetroot lip balm is made with natural, biodegradable ingredients, making it a better choice for the environment than synthetic lip balms that may have harmful chemicals^[6]

4.1. NOMENCLATURE OF BEETROOT

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Order: Caryophyllales

Family: Amaranthaceae

Genus: Beta

Species: B. vulgaris



Fig 2: Beetroot

4.2. USES OF BEETROOT LIPBALM

- It is used as a coloring agent.^[7]
- It acts as a binder.
- It gives a glossy appearance to the lips.
- It provides emollient action on the lips.
- It helps prevent cracking of the lips.

5. MATERIALS AND METHODS

5.1. UV Spectroscopy

Selection of λ_{max} :

- Accurately weighed 0.1g of methyl paraben and transferred it into a 250mL volumetric flask, then made up the volume to the mark with ethanol.
- Took 5mL from the stock solution and transferred it into a 100mL volumetric flask, then made up the volume with ethanol.
- Scanned the resulting solution from 200-400 nm using a 1cm pathlength to determine the λ_{max} .
- The same procedure was followed for two formulations: Dale View Naturals and Mamaearth lip balm.

Determination of Methylparaben

The presence of methyl paraben in lip balm samples was evaluated using UV-visible spectroscopic and HPLC methods.

Preparation of Standard Methyl Paraben:

To prepare the standard methyl paraben solution, 0.1g of methyl paraben was accurately weighed and transferred into a 250mL volumetric flask, then made up to the mark with ethanol. Next, 5mL of the stock solution was taken and transferred into a 100mL volumetric flask, and the volume was made up with ethanol. Serial dilutions were then prepared by transferring 1mL, 1.5mL, 2mL, 2.5mL, and 3mL of the stock solution into 10mL standard flasks, with the volume made up to the mark using ethanol.

5.2. HPLC

Stock solution:

To prepare the stock solution, 0.5g of methyl paraben was weighed and transferred into a 100mL volumetric flask, dissolved in 50mL of ethanol, and the volume was made up to 100mL. An acetonitrile-water mixture in a 70:30 ratio was then prepared and shaken for 5 minutes to be used as the mobile phase. Finally, 10 µL of the standard solution was injected into the HPLC column.

Sample preparation:

For sample preparation, 1g of the sample was weighed and transferred into a 100mL volumetric flask, and the volume was made up to the mark with ethanol. The same acetonitrile-water mixture in a 70:30 ratio was prepared and shaken for 5 minutes as the mobile phase. Then, 10 µL of the sample was injected into the HPLC column.

5.3. Antioxidant study

The DPPH method is used to determine the antioxidant properties of our beetroot lip balm formulations.

Procedure for DPPH Assay:

- **Preparation of Stock Solution:** Ascorbic acid was used as the reference standard. A stock solution of ascorbic acid was prepared in distilled water at a concentration of 1 mg/mL (w/v).
- **Preparation of DPPH Solution:** A 60 µM solution of DPPH in ethanol was freshly prepared.
- **Mixing the Sample:** 200 µL of the DPPH solution was mixed with 50 µL of the test sample at various concentrations (1.56, 3.12, 6.25, 12.5, 25, 50, 100, 200, 400, 800, and 1000 µg/mL).
- **Incubation:** The mixture was kept in the dark for 15 minutes at room temperature.
- **Measurement:** The decrease in absorbance was measured at 515 nm using a spectrophotometer.
- **Control:** The control was prepared with only DPPH solution, without any extract or ascorbic acid. 95% ethanol was used as the blank.

Calculation of Radial Scavenging Activity:

The percentage inhibition of DPPH radical was calculated using the formula:

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

5.4. Evaluation of Lip Balm:

i. Organoleptic Characteristics:

The formulation was examined for physical appearance, colour, and odour. The presence of coarse particles and consistency were assessed to evaluate the texture and homogeneity of the formulations.

ii. pH Test:

0.1g of the sample was mixed with 10mL of water. The mixture was filtered, and the pH of the filtrate was measured using a pH meter.

iii. Spreadability Test:

The sample was applied repeatedly onto a glass slide to visually observe the uniformity in the formulation of the protective layer and to check whether the lip balm was fragmented or deformed during application.

iv. Stability Studies:

The prepared lip balm was stored at room temperature ($25.0 \pm 3.0^{\circ}\text{C}$) for 30 days as part of accelerated stability studies. After 30 days, the lip balm was evaluated for its organoleptic properties, spreadability, and pH.

6. RESULTS AND DISCUSSIONS

6.1. UV Spectroscopy

Determination of λ_{max} in methyl paraben

The standard methyl paraben showed its λ_{max} at 257 nm.

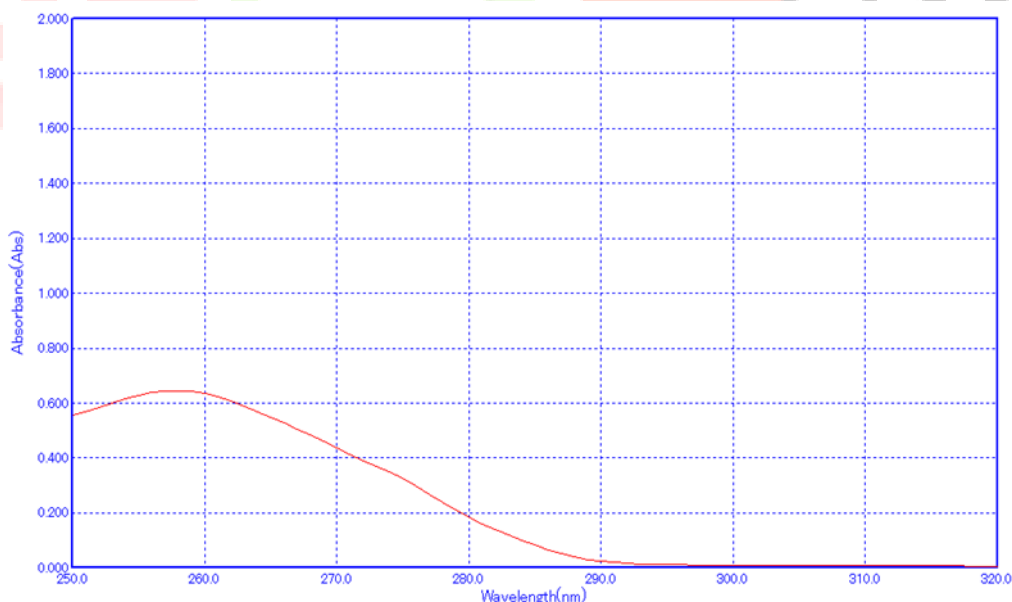


Fig 3: λ_{max} of standard Methyl paraben

Determination of λ max of methyl paraben in samples

(a) In the Dale View Naturals lip balm, no peak was observed at 257 nm.

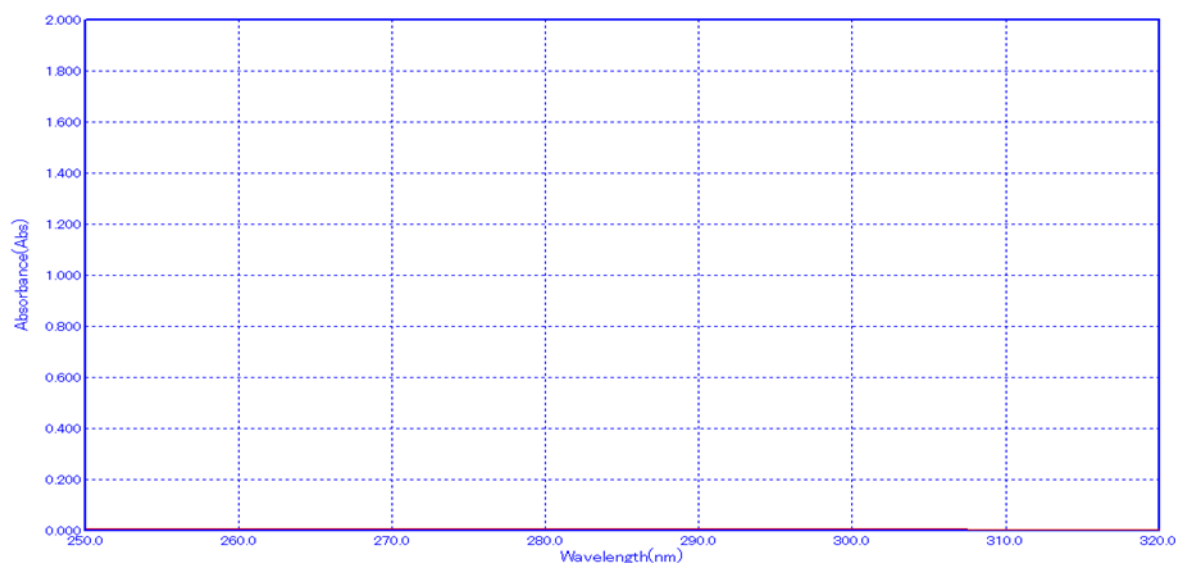


Fig 4: λ max of Dale View Naturals

(b) In the Mamaearth lip balm, no well-defined peak was observed at 257 nm.

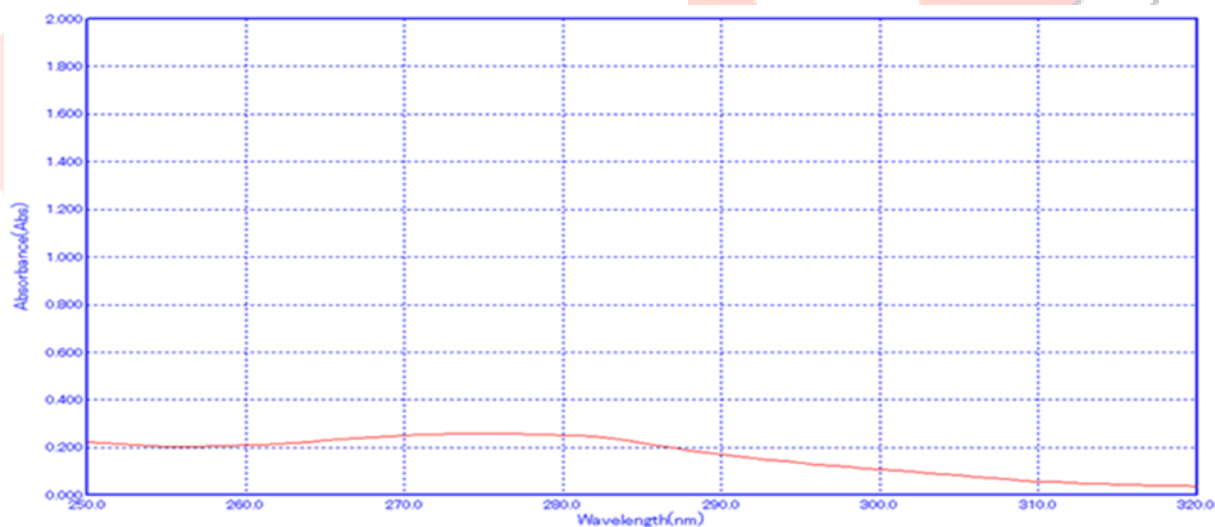
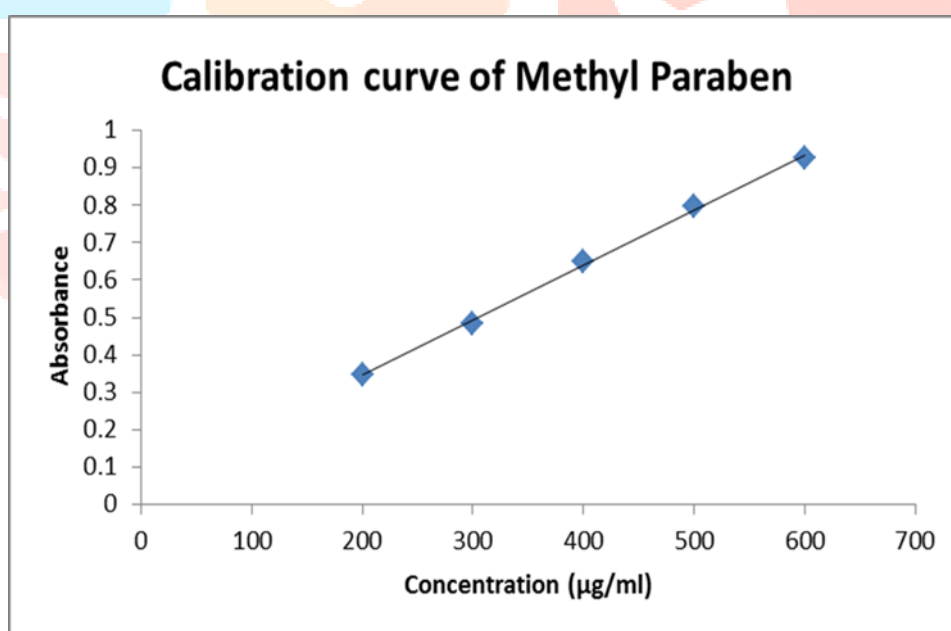


Fig 5: λ max of Mamaearth

Calibration curve of standard methylparaben:

CONCENTRATION ($\mu\text{g/ml}$)	ABSORBANCE (nm)
200	0.346
300	0.485
400	0.648
500	0.798
600	0.925

Table no: 1**Fig 6: Calibration Curve of Standard Methylparaben**

6.2. HPLC

The HPLC method confirmed that the samples contained methyl paraben by comparing them with the standard methyl paraben.

(a) Dale View Naturals lip balm showed a retention time between 3.4 and 3.8

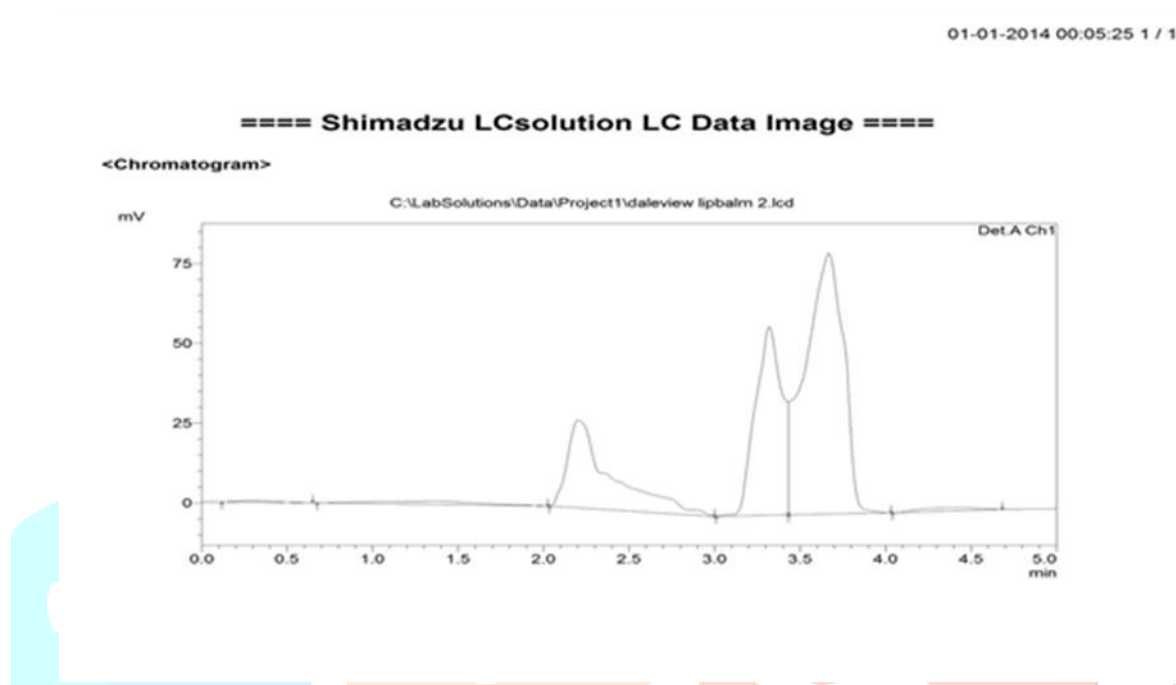


Fig 7: HPLC graph of Dale View Naturals

(b) Mamaearth lip balm showed a retention time between 3.2 and 3.6.

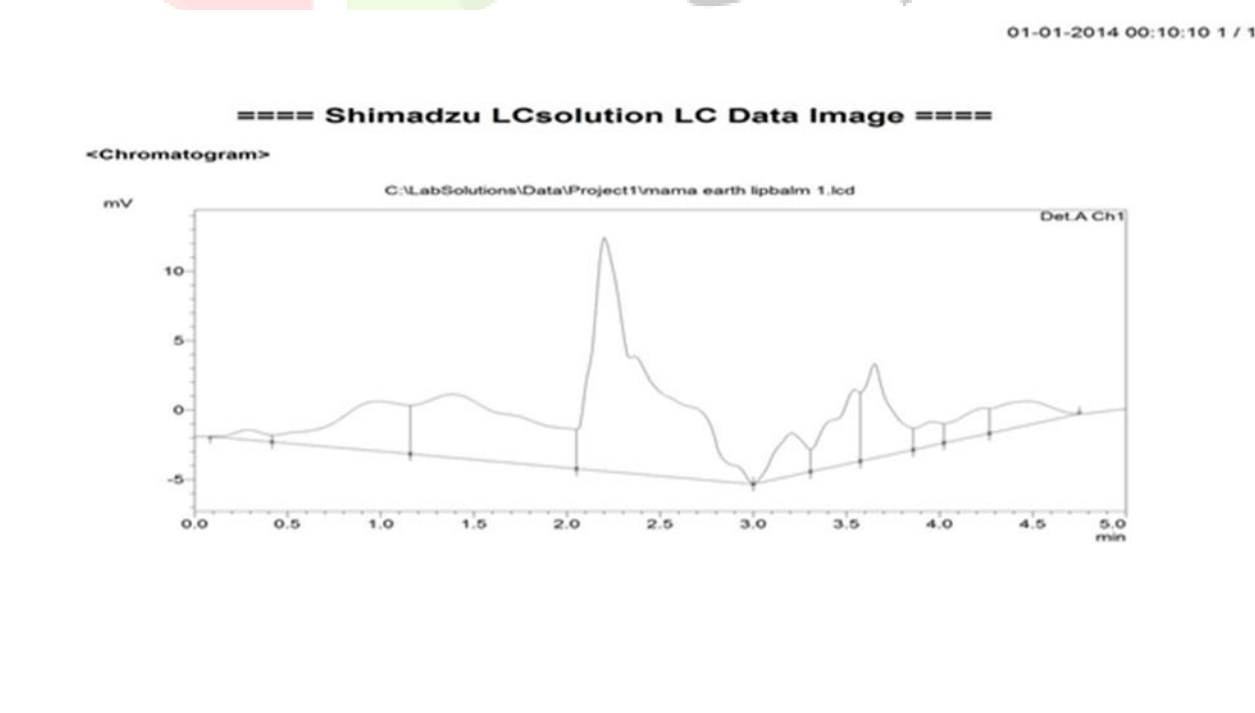


Fig 8: HPLC graph of Mamaearth

6.3. Anti-oxidant study

The DPPH assay revealed that Dale View Naturals, Mamaearth, and beetroot powder extract had the least antioxidant activity, as indicated by high absorbance values. In the beetroot lip balm, beetroot is included mainly for pigmentation rather than its antioxidant properties. Therefore, the low antioxidant activity reflects its role as a colorant rather than an antioxidant.

(a) Ascorbic acid

Standard	Concentration (µg/ml)	Absorbance at 515nm			Triplicate Average	Percentage Inhibition (%)
		OD1	OD2	OD3		
Control	-	0.8231	0.8226	0.8229	0.8229	-
Ascorbic acid	1.56	0.7987	0.7989	0.7881	0.7952	3.36
	3.12	0.7272	0.7274	0.7275	0.7274	11.61
	6.25	0.6813	0.6810	0.6813	0.6812	17.22
	12.5	0.5518	0.5514	0.5514	0.5515	32.98
	25	0.4585	0.4588	0.4588	0.4587	44.26
	50	0.1465	0.1466	0.1466	0.1466	82.19
	100	0.0926	0.0929	0.0922	0.0926	88.75
	200	0.0841	0.0848	0.0842	0.0844	89.75
	400	0.0654	0.0648	0.0647	0.0650	92.11
	800	0.0418	0.0411	0.0417	0.0415	94.95
	1000	0.0417	0.0415	0.0415	0.0416	94.95
	IC 50					28.19

Table no: 2

(b) Mamaearth

Sample code	Concentration (µg/ml)	Absorbance at 515nm			Triplicate Average	Percentage Inhibition (%)
		OD1	OD2	OD3		
Control	-	0.863	0.869	0.861	0.864	-
ME	1.56	0.852	0.852	0.859	0.854	1.12
	3.12	0.843	0.849	0.842	0.845	2.24
	6.25	0.803	0.809	0.802	0.805	6.87
	12.5	0.763	0.769	0.762	0.765	11.50
	25	0.733	0.738	0.735	0.735	14.89
	50	0.703	0.709	0.715	0.709	17.94
	100	0.693	0.694	0.691	0.693	19.83
	200	0.679	0.670	0.679	0.676	21.76
	400	0.652	0.654	0.658	0.655	24.23
	800	0.642	0.648	0.643	0.644	25.42
	1000	0.621	0.628	0.624	0.624	27.74
	IC 50*					<1000 µg/ml

Table no: 3

(c) Dale View Naturals

Sample code	Concentration ($\mu\text{g/ml}$)	Absorbance at 515nm			Triplicate Average	Percentage Inhibition (%)
		OD1	OD2	OD3		
Control	-	0.863	0.869	0.861	0.864	-
DV	1.56	0.826	0.829	0.821	0.825	4.48
	3.12	0.806	0.800	0.805	0.804	6.98
	6.25	0.772	0.779	0.773	0.775	10.34
	12.5	0.758	0.754	0.753	0.755	12.62
	25	0.713	0.718	0.712	0.714	17.32
	50	0.699	0.692	0.697	0.696	19.44
	100	0.652	0.659	0.654	0.655	24.19
	200	0.637	0.635	0.634	0.635	26.47
	400	0.613	0.618	0.612	0.614	28.90
	800	0.596	0.594	0.598	0.596	31.02
	1000	0.579	0.572	0.574	0.575	33.45
	IC 50*					<1000 $\mu\text{g/ml}$

Table no: 4

(d) Beetroot extract powder

Sample code	Concentration ($\mu\text{g/ml}$)	Absorbance at 515nm			Triplicate Average	Percentage Inhibition (%)
		OD1	OD2	OD3		
Control	-	0.875	0.875	0.873	0.874	-
BP	1.56	0.825	0.829	0.825	0.826	5.45
	3.12	0.782	0.781	0.789	0.784	10.30
	6.25	0.763	0.759	0.752	0.755	13.65
	12.5	0.700	0.709	0.702	0.704	19.49
	25	0.682	0.689	0.681	0.684	21.74
	50	0.652	0.654	0.659	0.655	25.06
	100	0.638	0.639	0.634	0.637	27.12
	200	0.611	0.618	0.612	0.614	29.79
	400	0.593	0.598	0.594	0.595	31.92
	800	0.557	0.552	0.555	0.555	36.54
	1000	0.513	0.519	0.524	0.519	40.66
	IC 50*					<1000 $\mu\text{g/ml}$

Table no: 5

7. EVALUATION

1. Organoleptic Characteristics:

The formulation was evaluated for its physical appearance, colour, and odour. The results of the samples are tabulated in Table No: [6].

2. pH Test:

The pH values of both lip balm formulations were found to be 7.3 for Dale View Naturals lip balm and 6.6 for Mamaearth lip balm, as shown in Table No: [6].

3. Spreadability:

The product was applied repeatedly onto a glass slide and was visually observed to remain intact, without fragmentation or deformation during application, as shown in [Figure No: 9 and 10].



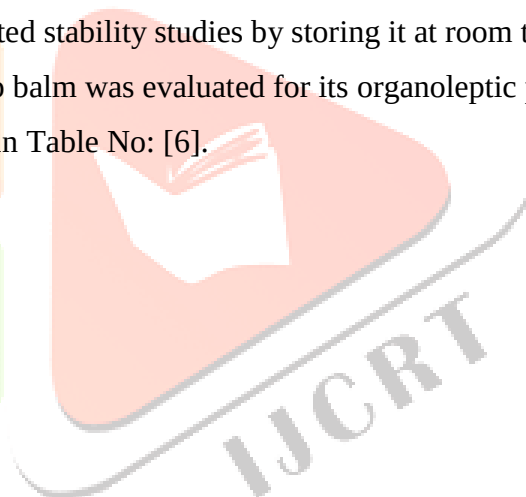
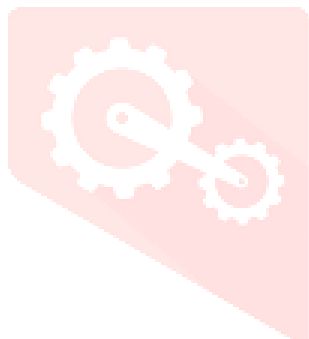
Fig 9: Spreadability of Dale View Naturals



Fig 10: Spreadability of Mamaeearth

4. Stability Testing:

The prepared lip balm was subjected to accelerated stability studies by storing it at room temperature ($25.0 \pm 3.0^{\circ}\text{C}$) for 30 days. After 30 days, the lip balm was evaluated for its organoleptic properties, spreadability, and pH. The results are tabulated in Table No: [6].



SL.NO.	EVALUATION PARAMETER	OBSERVED VALUE	
		Dale View Naturals	Mamaearth
1	Colour	pink	pink
2	Odour	pleasant	pleasant
3	Appearance	smooth	creamy
4	Spreadability test	good	good
5	pH	7.3	6.6
6	Stability study (25.0±3.0°C)		
a	colour	pink	pink
b	odour	pleasant	pleasant
c	appearance	smooth	creamy
d	spreadability	good	good
e	pH	7.3	6.6
f	Stability study	Stable	Stable

Table no: 6

8. CONCLUSION

From these results, we can conclude that:

- The UV spectroscopic method did not show any peak for the presence of methyl paraben in Dale View Naturals lip balm. In the case of the marketed product, Mamaearth, the UV spectroscopic method did not show a well-defined peak for the presence of methyl paraben.
- However, by HPLC, the presence of methyl paraben was detected in both samples.
- The DPPH assay showed that Dale View Naturals, Mamaearth, and beetroot powder extract had the least antioxidant properties.
- In the evaluation of physical parameters, the organoleptic properties were stable, and the spreadability was good. Regarding pH, the pH value for Dale View Naturals was found to be 7.3, which is higher than the given limit (around 6.6), whereas the marketed product, Mamaearth lip balm, showed a pH value of 6.6 and was within the acceptable range.

- In the stability study, Dale View Naturals lip balm was found to be stable at room temperature for 30 days.

9. ACKNOWLEDGMENT:

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10. CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

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