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Formulation and Evaluation of Herbal Lipbalm from Amaranth Leaf Colour Pigment

Yadav A.[1], Karmokar K.[2], Gop R.[3], Mudartha D.[4], Maheshwari V.[5]
[1, 2, 3, 4, 5]*Department of Biotechnology, Bhavans College, Mumbai University*

Abstract: ***Lips are soft and delicate and so are very prone to damage by drying, scaling, chapping. Lipbalms can provide protection to lips from these damages. Some organic colorants used to tint the lipbalms can be hazardous to health. Also, there is a shift from synthetic to herbal products for skin care. Considering these, this herbal lipbalm was made up and coloured by pigment extracted from Amaranthus dubius leaf colour pigment additive and many other ingredients which was evaluated in many parameters like pH, DPPH, melting point, aging stability, perfume stability, surface anomalies, etc... According to the survey conducted on volunteers, the colour, odour and visual appearance of the lipbalm was found to be very good, excellent and very good respectively. The consistency and glossiness were found to be excellent and the spreadability of the lipbalm was found to be very good. There was no irritation to any volunteer after applying the lipbalm.***
Keywords: Amaranthus dubius, lip, lipbalm, organic colorant, colour pigment.

I. INTRODUCTION

Lips, the visible body part at the mouth of humans and many animals, are soft, movable, and serve as the opening for food intake and in the articulation of sound and speech. Colouring lips is been practiced over years since the ancient period. Some [synthetic lip colorant, cheap and easy to make and obtain, can harm the lip skin. Also, lips do not contain any oil gland and therefore need a hydration and protection in all seasons [7]. In present day, lipbalm usage has been increased and also the colour shades and other choices have been changed. More often the applied lipbalm is eaten away and ingested and therefore it becomes mandatory for the health regulators to approve them with caution. Lipbalms can be used for colouring as well as moisturizing the lips. Herbal, is a sign of safety, satisfaction and surety of less or no harm to the users and so herbal lipbalm can be made without the colours being compromised on [4]. Amaranthus dubius leaves are high in nutrition value as well as it is also a promising crop. Its reddish-violet colour of the leaves owes to the presence of betacyanin in them [8]. Though the extracted colour pigments are susceptible to light and air, its stability could be maintained by keeping them in low temperatures (≤14°C) and also devoid from light and air. These can then be a potent organic colour pigment which can be used in food as well as cosmetic industries stating the stability conditions [9]. Coconut oil used for the formulation of this herbal pigmented lipbalm is exploited for its blending properties with the waxes and imparting the lipbalm to coat in thin film [12]. The beeswax could retain the moisture necessary for healing the dried chapped scaly lips and to maintain its quality [1]. The paraffin wax was used in very minute quantity to provide the gloss and rigidity to the lipbalm [1]. Emulsifier like polysorbate 80 (tween 80) was used for mixing the oil phase and water phase of the lipbalm and to provide a good concoction [34]. The lemon juice imparts to the lipbalm its bioactive and antioxidant properties [14]. The ginger juice was used for its antimicrobial and antifungal activities [15]. The strawberry essence and orange essential oil [16] were used to flavor the lipbalm and the nutmeg essential oil was used to provide its soothing and preservative properties including the antimicrobial and antifungal properties to the lipbalm [16] [17].

II. MATERIAL AND METHODS

A. *Selection of Material*

Amaranthus dubius (Lal math) and other materials used in herbal formulation were selected on the base of literature survey.

B. *Collection of Materials*

Materials used in formulation were procured from various different locations of Mumbai such as *Amaranthus dubius* (lal math) and *Zingiber officinale* (ginger) were procured from the local market of Kandivali, (Mumbai), *Citrus limon* L. (Lemon) was procured from the local market of Andheri, Mumbai, *Myristica fragrans* (Nutmeg) essential oil (Dr. JAIN'S), bee wax, coconut oil (Merit VCO Extra Virgin Coconut Oil) were procured from D. G. Ayurvedic Sangrah, Andheri, Mumbai, Paraffin wax, Tween 80 was procured from Science House (Ghatkopar west),Mumbai by the college, Strawberry essence was procured from A.I. Attarwala shop of Andheri west, Mumbai and *Citrus sinensis* (orange) essential oil was bought from Amazon.in

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C. Processing of Materials: (Modified [19])

1) *Preparation of Color pigment:* The leaves of *Amaranthus dubius* were separated from the stem and washed thoroughly with water and cleaned and Coloring pigment was obtained from the fresh leaves of *Amaranthus dubius* by mincing them in mortar-pestle and then squeezing and straining them through clean muslin cloth.
2) *Preparation of Ginger extract:* Ginger was peeled off and washed thoroughly with water and minced in mortar-pestle and then was squeezed and strained through clean muslin cloth.
3) *Preparation of Lemon extract:* Lemon was washed with water and also presterilized (wiped with 70% alcohol) before getting it in the laminar air flow then it was squeezed and strained through clean muslin cloth.

D. Preparation of herbal lip balm: (Modified [19])

The herbal lip balm was formulated as per general method of formulation in which the bee wax and paraffin wax were melted in porcelain dish on water bath with the decreasing order of melting point; coconut oil with tween 80 were heated in other porcelain dish then both the phases were mixed in the same temperature. Fresh ginger extract and fresh lemon juice were heated together in another porcelain dish (just before mixing). All the contents were mixed at 40^0C with the extract of leaves of *A.dubius*, the essential oils and essence are added drop wise with continuous stirring. The mixture was transferred from water bath to ice-bath and was allowed to solidify and then taken out of the ice-bath for further homogenization in mortar-pestle to get the desired texture. (Refer Fig.1.). The homogenized herbal lip balm was kept in mold and kept under refrigeration condition. Based on the protocol described above, formulation 1 (F1) (Refer Fig .2) and formulation 2 (F2) with following composition were prepared (Refer Table 1.)

Table 1. Formulation of Herbal Lipbalm

Sr no.	Ingredients	Quantity for Formulation 1 (F1)	Quantity for Formulation 2 (F2)	Importance
1.	Bees wax	3g	3.5g	Hardness and Glossiness
2.	Paraffin wax	3g	3.5g	Hardness and Glossiness
3.	Coconut oil	7ml	7ml	Blending property
4.	Tween 80	2ml	2ml	Surfactant
5.	Lemon juice	1ml	1ml	Anti-oxidant property
6.	Ginger extract	0.66ml	0.66ml	Anti-microbial property
7.	*Amaranthus dubius* extract	5ml	5ml	Colouring agent
8.	Strawberry essence	3drops	3drops	Flavouring agent
9.	Orange essential oil	3drops	3drops	Flavouring agent
10.	Nutmeg essential oil	2drops	2drops	Preservative

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Fig. 1 Formulated lipbalm during homogenization



Fig. 2 Formulated lipbalm in mold

E. Determination of Antioxidant Activity Using the 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Method (Modified [18])

The antioxidant property of *Amaranthus dubius* extract (AEE) and formulated lip balm (both F1 and F2) was estimated using DPPH assay method separately, where ascorbic acid used as standard commonly. Reagent and apparatus used are, DPPH reagent 0.01% of total concentration, Ascorbic acid 10µg/ml of total concentration, *Amaranthus dubius* extract 5mg/ml of total concentration, Formulated lip balm 500mg/ml of total concentration.

For standard from Ascorbic acid 10µg/ml of total stock solution various concentration ranging from 1-10µg/ml was prepared using distilled water as diluents as well as blank was prepared using 1ml of ethanol and followed by adding 1ml of DPPH regent in test tube then incubated at room temperature in dark for 20 minutes. Then absorbance measured at 593nm using colorimeter.

For DPPH assay of *Amaranthus dubius* extract various concentration ranging from 1000-3000µg/ml was prepared from 5mg/ml of total stock solution using distilled water as diluents as well as blank was prepared using 1ml of ethanol and followed by adding 1ml of DPPH regent in test tube then incubated at room temperature in dark for 20 minutes. Then absorbance measured at 593nm using colorimeter.

For DPPH assay of formulated lipbalm (both F1 and F2) same procedure was followed as mention above only the concentration ranging from 5-10µg/ml from total stock of 500mg/ml was prepared. Then absorbance measured at 593nm using colorimeter.

Then percentage (%) of radical scavenging activity was calculated for Standard, AEE and formulated lipbalm using the formula,

$$\%\text{Radical Scavenging Activity} = \frac{(\text{Absorbance of blank} - \text{Absorbance of sample})}{(\text{Absorbance of blank})} \times 100$$

Finally, the calibration curves obtained from different concentration of the Standard, AEE and formulated lipbalm extracts from which the IC50 (inhibitory concentration 50%) can be determined. IC50 value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals.

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F. Evaluation of herbal lipbalm

To achieve uniform standard of formulated herbal lip balm it is important to carry out its evaluations therefore, formulated herbal lip balm was evaluated through various parameter like melting point, spreadability test, perfume stability, etc.

1) *Melting point:*[19] Melting point determination of formulated herbal lip balm is important as it is an indication of the limit of safe storage and was determined by capillary tube method. In which the capillary was filled with formulated lip balm that was sealed at one end, then filled with lip balm and tied to a calibrated thermometer. The capillary along with thermometer was dipped in paraffin wax and was heated where temperature on the thermometer was noted when the lip balm in the capillary melted. The procedure was repeated 3 times and the melting point was determined by taking the average of 3 readings.
2) *Spreadability Test:* (Modified [20], [21]) Spreadability test done to estimate the force of application that is the force applied on lip balm to apply. In which parallel-plate method is the most widely used method for determining and quantifying the spreadability of semisolid preparations. The advantages of this method are simplicity and relative lack of expense. Steps followed in this evaluation are Sample was applied between two glass slides and was compressed to uniform thickness then weight was also added to the pan. The time required to separate the two slides, i.e. the time in which the upper glass slide moved over the lower slide was taken as measure of spreadability. Qualitative as well as quantitative estimation can be done, for quantitative the basic formula to be used given below.

$$\text{Spreadability} = \mathbf{m*l/\ t}$$

Where,

m = mass of lip balm applied between the slides.

l = length of spread by the glass slide.

t = time taken to spread.

3) *Surface Anomalies:* [20] This was studied for surface defects on formulated lip balm such as no formation of crystals no contamination by molds, fungi, etc.
4) *Aging Stability*: [20] The stability of product was evaluated by storing at 40^0C for 1 hr. Then various parameters such as bleeding, crystallization of on surface and ease of application were observed.
5) *Solubility Test:* [20] The solubility of formulated herbal lip balm was estimated by dissolving in various organic solvent like ethanol, chloroform, etc.
6) *pH parameter:*[21],[23],[24] The pH of formulated herbal lip balm was determined using pH meter. The pH meter was calibrated using buffer solution. Then 0.5 gm of lip balm was weighed and dissolved in 40ml of chloroform and its pH was measured.
7) *Skin Irritation Test:* [21] It is carried out by applying small amount of formulated product on the dorsal surface of left-hand skin for 10 minutes of 56 participated voluntary candidates. Then any kind of inflammation, rash, erythema, edema on skin examined.
8) *Perfume Stability:*[25] The formulated herbal lip balm was stored in standard storage condition of cool temperature then it was tested for its fragrance after 30 days.
9) *Acceptance of product:* Both the formulation of herbal lip balm acceptance was examined by 56 voluntarily participated candidates.

Table 2. Evaluation Chart of Herbal Lipbalm

Sr. No.	Evaluation parameter	F1	F2
1	Colour	Pale Pink	Pale Pink
2	Melting point	72^0C	76^oC
3	Spreadability (qualitative)	Even	Even
4	Surface anomalies	No	No
5	Aging stability	Smooth & Pale Pink	Smooth & Pale Pink
6	Solubility	Chloroform	Chloroform
7	pH	5.46	5.38
8	Skin irritation test	No	No
9	Perfume stability	+++	+++
10	Consistency	Smooth and Non-Granulated	Smooth and Granulated

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III. RESULTS

The present study was undertaken to formulate natural lipbalm from colored pigment of *Amaranthus dubius*. The study involves two different lip balm formulation called F1 and F2 illustrated in Table 1., along with their evaluation like color, melting point, spreadability, surface anomalies, aging stability, pH, skin irritation test, perfume stability and results of this test are illustrated in Table 2.

A. DPPH Assay

DPPH is stable free radical, purple in colour, the intensity of which is measured at 593 nm. DPPH Assay was used to check the antioxidant properties of the herbal lipbalm formulated from the *Amaranthus dubius* leaf colour pigment and the *Amaranthus dubius Ethanolic* Extract (AEE). Ascorbic acid was used as standard.

1) *DPPH radical scavenging activity of Ascorbic Acid:* Ascorbic acid used as a standard was found to have **IC50 = 5.43 µg/ml** as per the value in Table 3.

Table 3. Result of DPPH Assay of Standard

Conc. Of Std. (µg/ml)	O.D. at 593nm	%Radical Scavenging Activity
1	1.39	6.08%
2.5	1.16	21.62%
5	0.86	41.89%
6	0.67	54.73%
7	0.55	62.84%
8	0.28	81.08%
9	0.22	85.14%
10	0.17	88.51%

2) *DPPH radical scavenging activity of Amaranthus dubius Ethanolic Extract* (*AEE*)*:* The *Amaranthus dubius* Ethanolic Extract (AEE) reduced DPPH to 2, 2-diphenyl-1-picryl hydrazine and as per the values of Table 4. and Fig. 3.; the AEE has IC50 =2500µg/ml or 2.5mg/ml.

Table 4. Result of DPPH Assay of AEE

Conc. of Sample. (µg/ml)	O.D. at 593nm	%Radical Scavenging Activity
1000	1.12	24.32%
1500	1.07	27.70%
2000	0.86	41.89%
2500	0.75	49.32%
3000	0.61	58.78%

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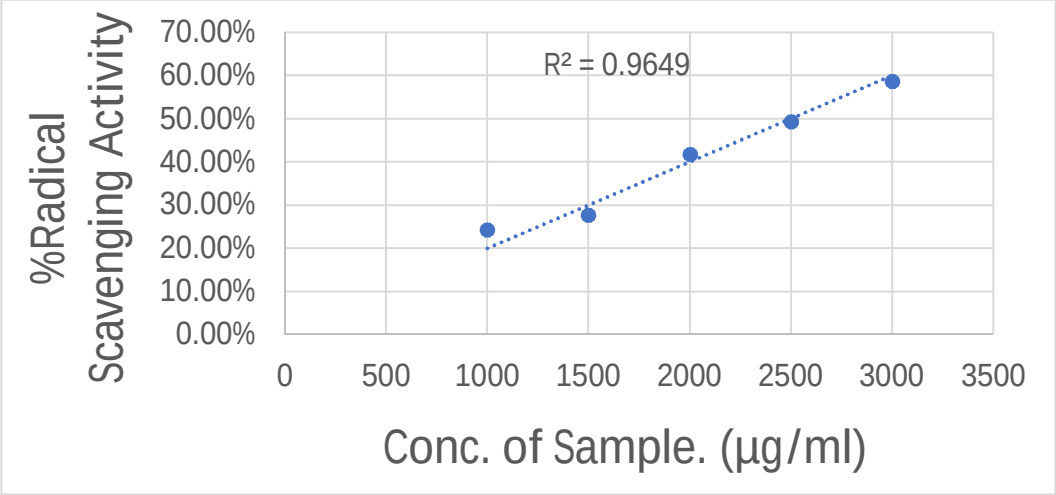


Fig. 3 DPPH assay sample

3) *DPPH radical scavenging activity of lip balm:* As per the values of Table 5. and Fig. 5; Herbal Lip balm used as a sample was found to have IC50 =250mg/ml

Table 5. Result of DPPH Assay of Herbal Lipbalm

Conc. of Sample. (mg/ml)	O.D. at 593nm	%Radical Scavenging Activity
50	1.66	10.75%
100	1.61	13.44%
150	1.18	36.56%
200	1.00	46.24%
250	0.98	47.31%
300	0.87	53.23%

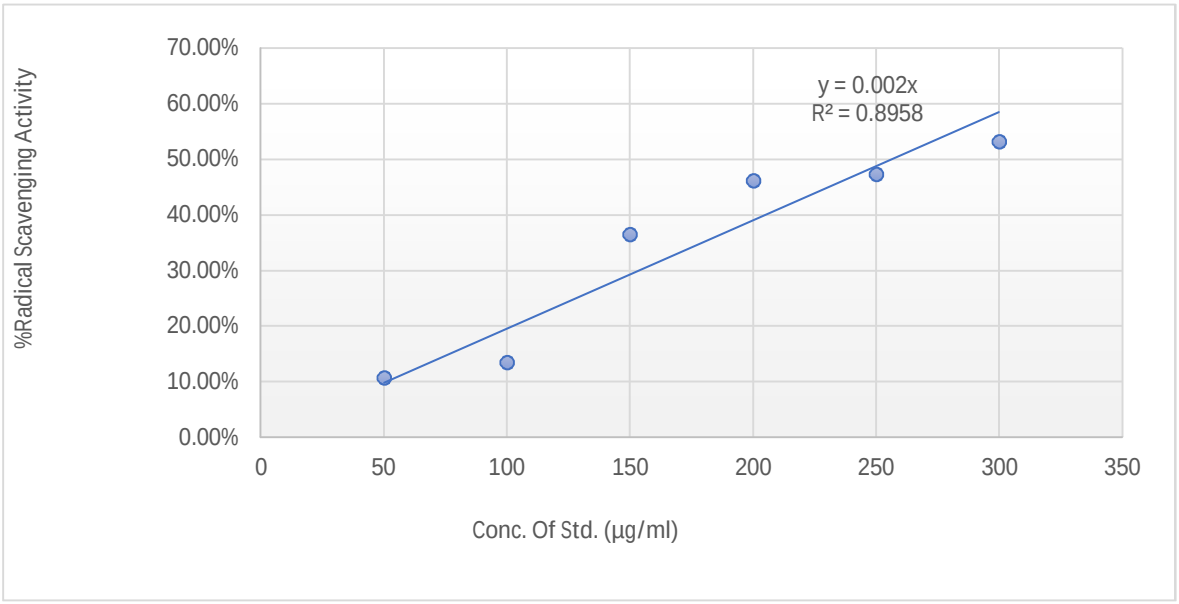


Fig. 4 DPPH assay of lipbalm

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4) *Epidemiological Survey* To check the efficiency and acceptance of formulated herbal lip balm epidemiologic survey was carried out on 56 voluntarily participated candidates. In this survey certain parameter was evaluated on bases ratings given by candidates are as follow: 1= Very Poor, 2= Fair, 3= Good, 4= Very Good, 5=Excellent. In which physical properties (colour and odour), homogeneity (appearance and consistency) and after application of formulated herbal lip balm feedback was taken and data obtained presented in form of graph are as given below.

5) *Physical Properties of Lip balm:* Color of formulated herbal lip balm found very good as graph shows maximum percentage around at 44.6%.

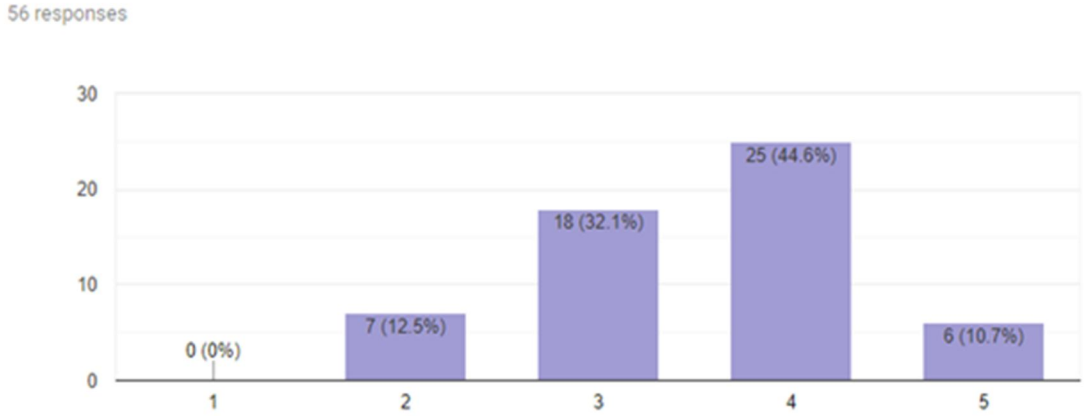


Fig. 5 Color of lipbalm

The respond for odour found excellent according to the feedback given and plotted on the graph and found maximum percentage that is 58.9%

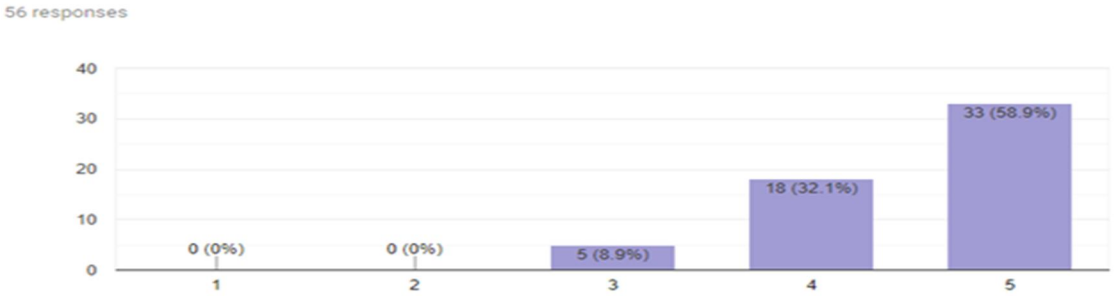


Fig. 6 Odour of lipbalm

6) *Homogeneity of Lipbalm:* The formulated herbal lip balm having visual appearance found very good because 46.6% of volunteer rated which is maximum as shown in the graph obtain after feedback.

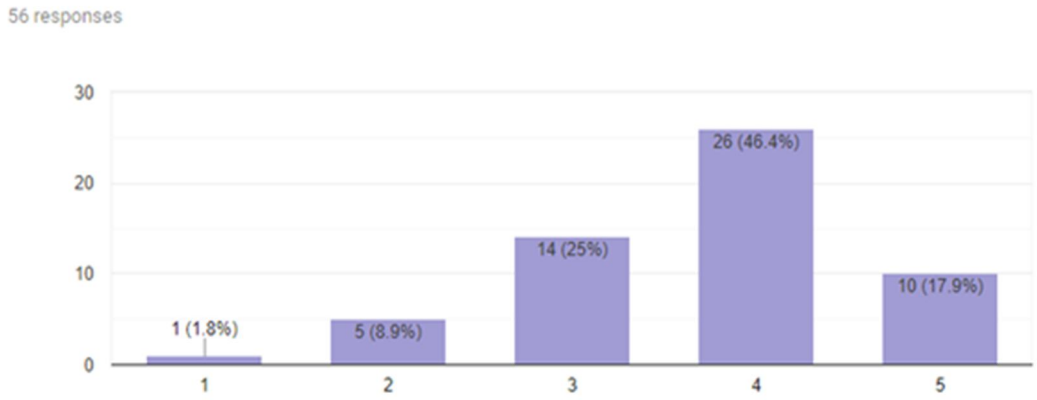


Fig. 7 Visual appearance of lipbalm

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7) *Consistency of Lipbalm*: While the consistency was Excellent as well as very good graded by maximum volunteer

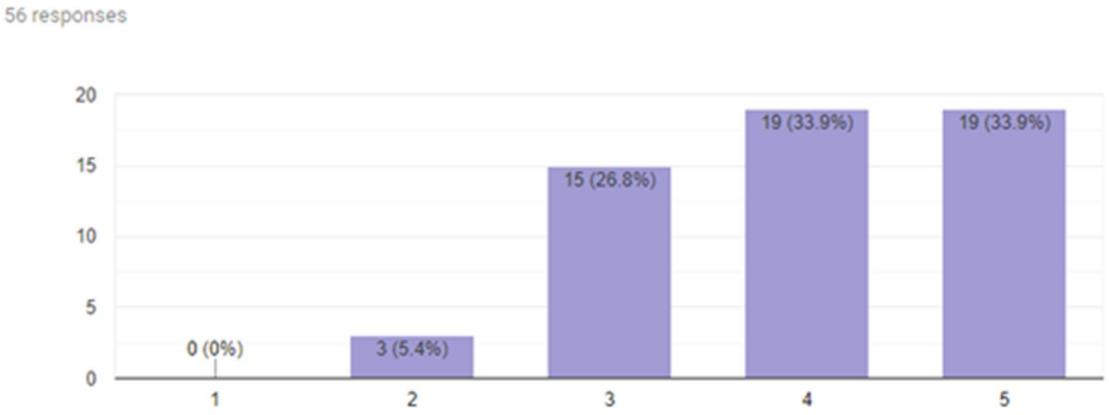


Fig. 8 Consistency of lipbalm

8) *Glossiness of Lipbalm:* In terms of glossiness the feedback was found to be very good

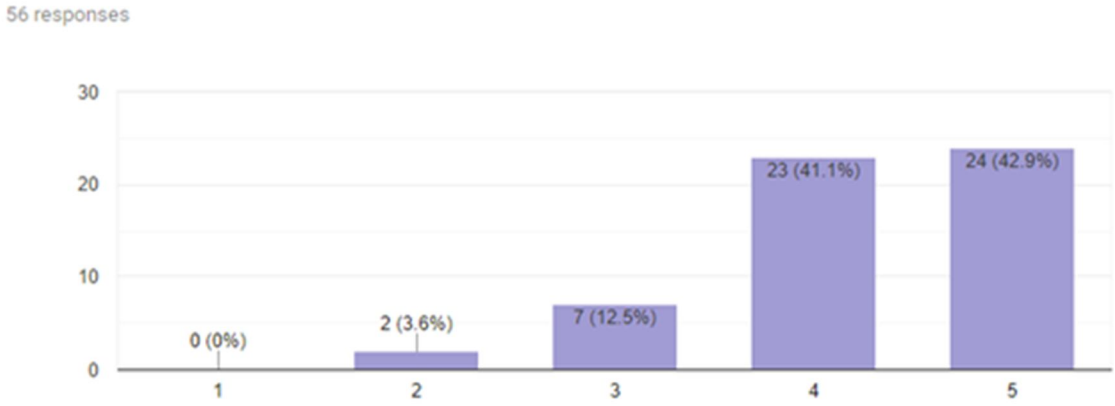


Fig. 9 Glossiness of lipbalm

9) *Irritancy Test:* No irritancy was found

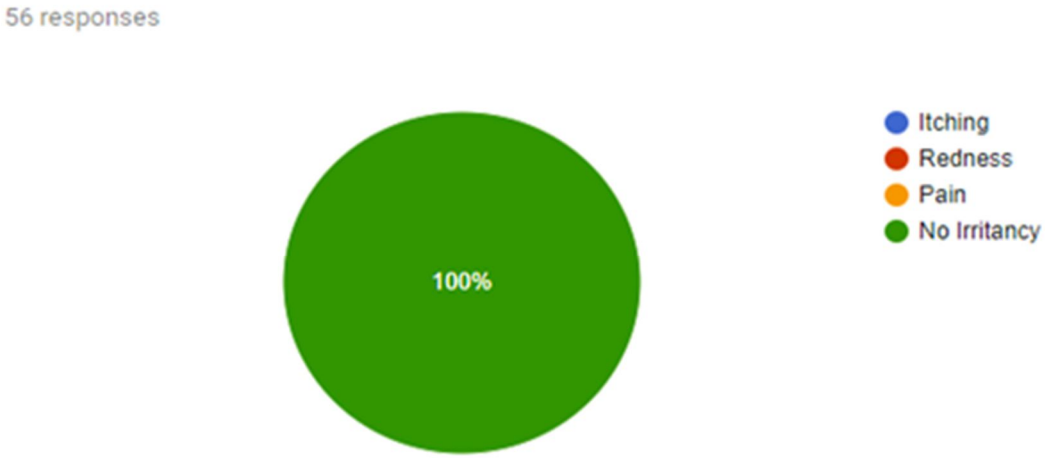


Fig. 10 Results of irritancy test

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IV. DISCUSSION

The present work is undertaken for formulation and evaluation of herbal lip balm with the hope to minimize the side effects of chemical colours. Plants pigments provide antioxidant properties along with the colouring property used in this herbal formulation, we have used both the properties of Amaranthus dubius plant and as the results obtained are the formulated herbal lip balm has antioxidant properties, estimated by DPPH assay for which ascorbic acid was chosen as standard which showed 50% radical scavenging activity at 5.43µg/ml and Amaranthus dubius extract showed similar activity at 2500µg/ml. Though extract scavenging activity is approximately 500-fold lesser than that of the standard but the source for the activity is due to naturally present pigment from Amaranthus dubius leaves. The formulated herbal lip balm shows 50% radical scavenging activity at 250mg/ml which showed approximately100-fold lesser 50% scavenging activity than the A.dubius extract. The antioxidant activity can be enhanced under stringent laboratory condition as this formulation was done at college pilot scale level and we may replace the chemical based synthetic colour and antioxidant by herbal based preparations. The formulated herbal lip balm had efficient melting and molding capacity which is successfully evaluated with various parameters. The colour of lip balm was pale pink. The pH of lip balm was found to be 5.46. Which was in the range of pH of skin therefore it signified that it is compatible for skin (lip) which was further confirm by skin irritation test done in survey on voluntarily participated candidates. The melting point of lip balm was in between 72^0c to 76^0c. It confirmed that the formulated lip balm might be stable at room temperature or even at comparative high temperature but below 72^0c and therefore melting temperature was acceptable. The surface anomalies had also not found. The force of application or spreadability was found even but further homogenization can be done for better results. The aging stability was also found smooth but for a shorter period of time and then the colour was further faded because stabilizer was not added. Solubility test was also performed and found soluble in chloroform. The skin irritation test showed no such sign of itching, irritation, redness, and inflammation. The overall work performed reveal that the formulated herbal lip balm was safe and compatible to skin when compared to previous studies. Further studies on the herbal lip balm can be done for enhancing antioxidant property and colour stability at room temperature.

To check the efficiency of formulated herbal lip balm epidemiologic survey was carried out on 56 voluntarily participated candidates. In this survey certain parameter was evaluated on bases ratings given by candidates are as follow: 1= Very Poor, 2= Fair, 3= Good, 4= Very Good, 5=Excellent. In which physical properties of formulated herbal lip balm like color, odour, visual appearance and consistency was rated as very good, excellent, very good and very good respectively. After application of lip balm on volunteers', the ratings found for lip balm was very good for spreadability, excellent for glossiness and also no irritancy found to any of the of the volunteers, therefore, the formulation was found to be safe for skin application.

V. CONCLUSION

The present work carried out the formulation and evaluation of herbal lipbalms was aimed to formulate a lip balm using herbal ingredients with an idea to minimize the side effects which occur by using available chemical based synthetic lip balms. The prepared formulation of lip balm was evaluated and it was found that the herbal lipbalm, based on the consistency, Formulation-1 was best among the two formulations. Therefore, from present investigation it was concluded that this formulated herbal lip balm provides a better option for anyone applying lip balm with minimal side effects and also have antioxidant properties which help for better health benefits. The further studies can be carried out on the basis of present study of formulation and evaluation of herbal lipbalm using Amaranthus dubius extract are as follow:

1) Various clinical trials can be done to check effect on skin and internal organs for detail study of formulated herbal lip balm.
2) Other method of formulation of herbal lip balm and extraction of pigment can be used to get more antioxidant property in lesser amount of pigment.
3) As pigment is stable below 14^0C, stabilizer can be use.
4) To enhance the even texture and smoothness one can go for better homogenizing method.
5) To get the various shades in formulated herbal lip balm the amount of pigment can increase and decrease accordingly.

VI. ACKNOWLEDGEMENT

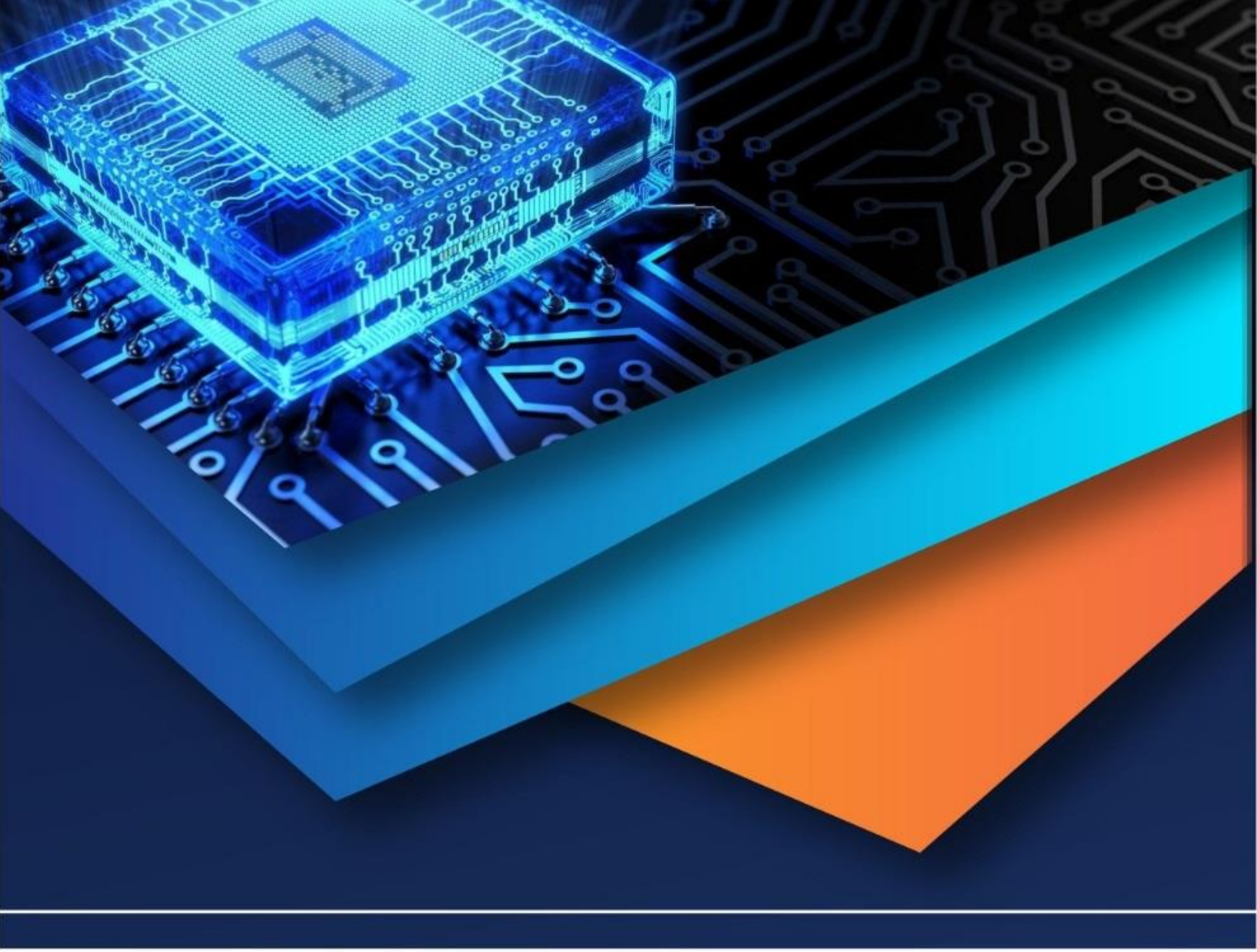
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