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Research Article

Formulation of Cosmetics using Extract of Teak Leaves (*Tectona grandis*) as a Colourant

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ABSTRACT

Beautification of skin and hair by dyeing it or by colouring lips, cheeks and eyes, have been a part of our living since ages. So, to avail this purpose, large number of shades were manufactured using chemicals, but, their frequent use showed allergies and eczema. This led to extraction and use of natural colorants. Thus, this project is about formulation and evaluation of herbal cosmetics- lip balm and shampoo, with the herbal ingredient being, colour extract obtained from dried leaves of Tectona grandis (Teak wood). The dye was extracted from the leaf powder by using maceration. The concentrated extract was subjected to solubility tests, UV-visible, TLC and phytochemical analysis. Lip balm was characterized for stability studies like organoleptic characters, melting point, water resistance, whereas shampoo was subjected to tests like rheological studies, bio assay, cleansing action. The concentrated extract showed good solubility and colour as hydro-alcoholic solution and was thus used in both the formulations. UV spectra and TLC showed presence of anthocyanin in hydro-alcoholic solution. Finest lip balm was formed after triturating the colorant with the waxy base. It showed stability at room temperature and in refrigerator and a melting point around 500C. The shampoo showed good results for stability, foam stability and bioassay, and solid content in the range of 16% -18%. Hence, a good aqueous and lipid based cosmetic can be formulated by replacing synthetic colour with teak leaves extract, which can produce different shades of colours, from light orange to dark brown.

Keywords: Cosmetics, *Tectona Grandis*, extract, shampoo

INTRODUCTION

India is a country which is known for its ethnicity and traditional way of lifestyle. Nature has played a crucial role in enhancing this way of living for Indians. Indians have also earned their livelihood and continue to do so even today, by the help of various assets of nature, out of which trees being the most valuable one. About 3,900 plant species are used as a source of food, fibre, fodder, and other species are used for extraction of gum, resins, and colorants. So, in this report we specifically focus on colorants procured from nature. Colours have always influenced Indian living and customs to a great extent; right from using it for dyeing and decorating clothes, furniture, food to beautifying oneself. Beautifying skin and hair by dyeing, or beautifying face by colouring lips, cheeks, eyes during special occasions have been a part of our living since ages.[1] Now, this concept has evolved from mere colouring to using cosmetics that offer infinite number of shades for beautification. Colorants used in cosmetics are of two types, and are classified on the basis of their manufacturing process; natural colorants (obtained naturally from plants) and synthetic colorants (synthesized from chemical reactions). Synthetic dyes have been widely available and in use since 1856.[2] They show excellent, long lasting dyeing properties, are easy to apply, easily produced and are available in wide range of shades. Most common synthetic dyes used in industry are alizarin, tartrazin, indigo, eosine. But recently, with the rise in use of cosmetics, various side effects of synthetic dyes are observed like mutagenicity, carcinogenic effects, allergies, eczema, rashes, organ toxicities, environmental pollution, which is mainly because of intense and toxic chemicals in them.[3] This, has led the way for herbal cosmetics [4] and skin care products which contain natural colorants that are manufactured free of chemicals and also, they are comparatively much safer since they do not cause any of the above side effects.[5] Thereby, also increasing the demand of natural colorants in market. [4] Thus, this project is based on formulation of herbal cosmetic formulations- lip balm and shampoo using naturally extracted colour from Jati leaves (Tectona grandis), which is one of the local resources that consist of natural pigment. The leaves have been collected from Konkan region of Maharashtra. Jati leaves (Tectona grandis) belong to family- Verbenaceae and consist mainly of pigment anthocyanin, which are responsible for the dyeing property. Anthocyanin give red, blue, purple, yellow, magenta shades, and is not toxic. Anthocyanin are found in plant cell vacuoles, are highly reactive and easily oxidizable. In addition of role of colouring it also exhibits antioxidant activity. [6] Anthocyanin exhibit polar properties hence, are dissolved in polar solvent such as methanol acidified with HCl, which proves to be most effective.[6] Due to the toxicity of methanol in high quantities, ethanol can be used to replace methanol.[6] But, methanol in small specified quantities is safe for use in cosmetics.[7] Hydrochloric acid

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in methanol will denature the cell and releases the pigment and dissolve it in the solvent outside the cell.[6] The extraction of colorant uses maceration as an extraction process, and two formulations were made by us using this natural colorant which is Lip balm and Shampoo.[4] Thus, the purpose of this study is to prove that jati leaves extract can be used as colorant in the formulation of these cosmetics.

MATERIALS AND METHODS

Plant material

Lemon oil (Research lab), Paraffin (Sd fine), Petroleum jelly (Sd fine), Beeswax (Sd fine/ Research lab), Sodium benzoate (Sd fine), Sodium chloride (Sd fine), Cocoa butter (Research lab), SLES (Research lab), Nutrient broth (High media laboratories pvt. Ltd.), Stalagmometer (Dr. U. B. Hadkar sir), Ph meter (ELICO LI IZO), Evaporator (META lab), Brookfield viscometer (Brookfield engg. laboratories U.S.A.), Thermometer, Soxhlet, Sodium Chloride (Nacl) solution, citric acid, Sonicator.

Solubility and Colour:

1. Solubility-The solubility was checked in three mediums – water, hydro-alcoholic mixture, paraffin oil. Mixing of colour concentrate with the oil medium was done using a cyclo mixer.
2. Colour-Colour was checked in three different medium non-pH adjusted hydro-alcoholic solvent, pH adjusted hydro-alcoholic solvent, water. [8,9]

Extraction: Extraction of natural dye from teak leaves was done using two different processes of extraction- soxhlet and maceration, and their results were compared.

1. Soxhlet-25 g. of leaf powder was sieved through #60 mesh and subjected to soxhlet extraction for 8 hrs. Solvent used for extraction was 125ml hydro alcoholic mixture (methanol:water ratio- 60:40).[10]
2. Maceration- Leaf powder was subjected to maceration in the ratio of 1:5 (1g of powder and 5g of solvent). (Extract obtained from both the methods were observed and soxhlet extract was selected.)

Analysis of Extract:

1. Thin Layer Chromatography (TLC)- Solvent systems are as follows:

a) Toluene: Ethyl acetate: Formic acid (5:4:1)
b) Chloroform: Methanol (9:1) (2 TLCs were performed using solvent system

2. Uv-vis spectra-1:50 dilution of pure extract was prepared using 60% methanol as solvent, and was used to run spectra. Wavelength selected was between 400-800nm. [9]
3. Phytochemical AnalysisFollowing tests were performed

a) STEROIDS- 1ml extract was dissolved in 10 ml of chloroform & equal volume of concentrated H2SO4 acid was added from the side of test tube result gives two layers, Upper layer showed red and lower layer yellow with green fluorescence, indicated positive test.[11]
b) TANNINS- 4 ml of Extract was reacted with FeCl3 solution gave green colour, indicating Positive test.[11]
c) SAPONIN – 20 ml of distilled water was added to 5 ml extract and shake well wait for 15 min, foam formation indicated positive test. [11]
d) COUMARIN - 3 ml of 10% NaOH was added to 2 ml of aqueous extract formation of yellow colour indicated coumarins. [11 7]
e) EMODINS-2 ml of NH4OH and 3 ml of benzene was added to extract appearance of red colour indicated presence of emodins. [11]
f) CARBOHYDRATES-Extract were dissolved in 5ml of distilled water and filtered. The filtrate was used for the Iodine test. Iodine Test: - 2ml of extract were treated with 5 drops of Iodine solution, gives blue colour indicated the positive test. [11]
g) DITERPENES-Copper acetate test: - Extract were dissolved in water and treated with 10 drops of copper acetate solution, formation of emerald green colour indicated presence of diterpenes. [11]
h) CARDIAL GLYCOSIDES – Keller-Killani Test: - Plant extract treated with 2ml glacial acetic acid containing a drop of FeCl3. A brown colour ring indicates the presence of positive test. [11]
i) CHALCONES-2ml of NH4OH was added to 0.5g ethanolic extract, appearance of red colour showed the presence of chalcones. [11]
j) PHENOL-Ferric Chloride test: - Test extract was treated with 4 drops of Alcoholic FeCl3 solution. [11]
k) PHLOBATANNINS-Deposition of red ppt. when aqueous extract of each plant sample is boiled with 1% Aqueous HCl was taken as evidence for presence of Phlobatannins.[11]
l) PROTEINS-Xanthoproteic test: Extract was treated with few drops of concentrated HNO3 formation of yellow indicates the presence of proteins. [11]
m) PHYTOSTEROL-Salkowski's test: - Extract was treated with chloroform and filtered. The filtrate was treated with few drops of concentrated H2SO4 and shakes, allow standing, appearance of golden red indicates the positive test. [11]
n) FLAVONOIDS-Alkaline reagent test: - Extract was treated with 10 % NaOH solution, formation of intense yellow colour indicated presence of Flavonoid. [11 8]
o) ANTHOCYANIN-2 ml of aqueous extract is added to 2 ml of 2N HCl & NH3, the appearance of pink red turns blue violet indicates presence of Anthocyanin. [11]
p) ALKALOIDS-A quantity (3 ml) of concentrated extract was taken into a test tube and 1 ml HCl was added the mixture was heated gently for 20 min cooled and filter, the filtrate was used for Following test. Wagner test: - 1ml of the extract was treated with Wagner's reagent; formation of brown reddish precipitate indicates presence of alkaloids. [11]
q) LEUCOANTHOCYANIN - 5 ml of isoamyl alcohol added to 5 ml of aqueous extract, upper layer appear red in colour indicates the presence of Leucoanthocyanin. 11

Formulation:

1. Formula table-

a) Lip balm 12–14 - Refer to table no. 1
b) Shampoo 14–16 - Refer to table no. 2

2. Formula-

a) Lip balm-Making of lip balm is majorly divided into two steps, out of which preparation of base is the first one followed by addition of colorant. Three different formulations of lip balm were made out of which the best preparation was selected. Preparation of base is common for all three formulations which is as followsIn a beaker, mentioned quantity of bees wax, cocoa butter, petroleum jelly and liquid paraffin was taken. Beaker was placed in a water bath. A thermometer was placed in the beaker and frequent mixing of the ingredients was done and oils were added. After the preparation of base, colorant was added. 9 Method for addition of colorant is different for all the formulations which is as follows-i. Formula 1- Weighed quantity of concentrated colorant was added into the beaker. It was filtered off through a muslin cloth into another beaker. The beaker was kept in the water bath allowing it to gradually cool down. ii. Formula 2 (method A)- Weighed quantity of concentrated colorant was triturated with methanol and added into the beaker. It was filtered off through a muslin cloth into another beaker. The beaker was kept in the water bath allowing it to gradually cool down. iii. Formula 2 (method B)- After the ingredients of base were mixed, they were allowed to set, forming lip balm without colorant. Then the set base was taken into mortar pestle, and was triturated with concentrated colour extract continuously. Then the coloured mixture was filtered off through a muslin cloth and transferred in vessel and allowed to set.

b) Shampoo-Four different formulations of shampoo were made under different conditions and better shampoo was selected out of them. (Method to prepare formula 1 and 2 is the same with preservative being the only point of difference. Refer the formula table for the same)

i. Formula 1 &2 – METHOD- Add the preservative in water and dissolve with the help of heat. Add SLES. (For pH adjusted) adjust the pH with citric acid solution (0.5gms of citric acid in 5ml of water) till pH gets to 3. Adjust the viscosity with Sodium Chloride. Add lemon oil and then add the colour extract solution. Again add Sodium Chloride till desired viscosity is attained. 10 ii. Formula 3, 4 – (Method to prepare formula 3 and 4 is the same with the amount of preservative being the only point of difference. Refer the formula table for the same) METHOD- Add the preservative in water till it dissolves. Add SLES in the same beaker. Adjust the viscosity with Sodium Chloride. Add lemon oil and then add the colour extract solution. Again add Sodium Chloride till desired viscosity is attained.

Product Evaluation:

1. Lip balm-a) Organoleptic propertiesMETHOD- Checking for colour, odour, consistency and shine. [12] b) Spreadability-METHOD- Place the lip balm at the centre of a glass slide, place another glass slide on top of it and place a weight of 50gms on it . Remove the weight after 2 mins. Lip balm spreads without any fragments and the diameter was noted. [12] c) Melting point/ Softening point METHOD- Fill the product in a capillary and introduce it in a water bath at room temperature and start heating it. Noted the temperature at which the product moves out of the capillary. [12] d) Water resistance-METHOD- Weigh the product and place it on a slide whose weight is known. Place another slide on it whose weight is also known and keep them that way for 1 min and then separate both the slides. Again weigh both the slides and note down the weight. Dip them in a beaker containing water for 2 mins. and then remove them. Dry the slides till all the water molecules get evaporated. Again weigh both the slides and note down the readings. Continue taking the readings till 10% weight loss is observed. [11] e) Microbial load METHOD- All the petri plates, media and other necessary apparatus was sterilized in autoclave prior to use. Media used was nutrient agar as we had to check bacterial growth. Sample was diluted in following manner: 1g of lip balm with 5ml with chloroform (solution A). 1ml of solution A with 0.9ml of saline (solution B). 0.1ml of solution B with 0.9ml of saline (solution C). 0.1ml of solution C was poured on to media plate and spread with glass spreader then kept for incubation for 24hrs. f) Temperature stabilityLip balm formulated by formula 2, method B was stored at room temperature, in incubator (temperature slightly higher than room temperature- 370C - 400C) and in refrigerator to check for stability.

2. Shampoo- a) Organoleptic properties-METHOD- Checking for colour, odour, and pH. [17] b) Foaming and foam stability-METHOD- Cylinder shake method was most widely used for determining foaming ability. 5ml of the 1% shampoo solution was put into a 50 ml graduated cylinder and volume was made up to 25 ml with distilled water covered the cylinder with hand and shaken for 10 times. The total volumes of the foam contents after 1 minute shaking were recorded. The foam volume was calculated. Immediately after that the volume of foam at 1 minute intervals for 4 minutes were recorded. [17] c) Dirt dispersion-METHOD- Two drops of shampoo were added in a large test tube contain 10 ml of distilled water. 1drop of India ink was added; the test tube was stoppered and shakes it ten times. The 12 amount of ink in the foam was estimated as None, Light, Moderate, or Heavy. Shampoos that cause the ink to concentrate in the foam are considered poor quality. The dirt should stay in the water portion. Dirt that stays in the foam will be difficult to rinse away. It will redeposit on the hair. [15,17] d) Determination of percentage solid content-METHOD - A clean dry evaporating dish was weighed and added 4 grams of shampoo to the evaporating dish. The dish and shampoo was weighed. The exact weight of the shampoo was calculated only and put the evaporating dish with shampoo was placed on the hot plate until the liquid portion was evaporated. The weight of the shampoo only (solids) after drying was calculated. If a shampoo has too many solids it will be hard to work into the hair or too hard to wash out. If it doesn't have enough it will be too watery and wash away quickly. A good shampoo will be between 20% – 30% solids. [15,16] e) Cleaning action-METHOD - 5 grams of wool yarn were placed in grease, after that it was placed in 200 ml. of water containing 1 gram of shampoo in a flask. Temperature of water was maintained at 350C. The flask was shaken for 4 minutes at the rate of 50 times a minute. The solution was removed and sample was taken out, dried and weighed. The amount of grease removed was calculated by using the

following equation DP = 100 (1-T/C) In which, DP is the percentage of detergency power, C is the weight of sebum in the control sample and T is the weight of sebum in the test sample. [17] f) Bioassay-METHOD- All the petri plates, media and other necessary apparatus was sterilized in autoclave prior to use. Nutrient agar broth was used as media since bacterial culture was used to check the activity of preservative. Liquid nutrient broth was poured onto plates and solidified. Culture was made using saline solution. Saline solution was poured into test tube 13 with grown bacterial culture. Kept for few minutes to transfer the bacteria into saline solution. This bacterial solution was then swabbed onto the media plate with help of cotton bud. Wells were made using cork borer. Wells were filled with shampoo solution. (Dilutions of shampoo solution made were 1:2 and 1:4 using distilled water). The plates were kept for incubation for 24 hrs. Next day plates were observed for zone of inhibition. [18] g) Microbial load-METHOD- All the petri plates, media and other necessary apparatus was sterilized in autoclave prior to use. Media used was nutrient agar as we had to check bacterial growth. Sample was diluted in following manner: 1g of shampoo with 5ml with chloroform (solution A). 1ml of solution A with 0.9ml of saline (solution B). 0.1ml of solution B with 0.9ml of saline (solution C). 0.1ml of solution C was poured on to media plate and spread with glass spreader then kept for incubation for 24hrs. h) Surface tension-METHOD- Number of drops for 1ml of shampoo was calculated using stalagmometer. Then surface tension was calculated using the formula:

ST= D*nw/n*Sw.[15,16] D= Density= w3-w1/w2-w1 (calculated using pycnometer) W1= weight of pycnometer W2= weight of pycnometer + distilled water W3= weight of pycnometer + formulation n w = no. of drops in 1ml of water n= no of drops of shampoo Sw = surface tension of water = 72 Dynes/cm. These were our final formulationsRefer to figure no. 1 for final Lip balm formulation & no. 2 for Shampoo.

Figure 1: Lip Balm

RESULTS AND DICUSSIONS

1. Lip balm-

a) Organoleptic properties- Refer to table no. 3 [12] b) Spreadability- Refer to table no. 4 [12] c) Softening (Melting) point- Refer to table no. 5 [12] d) Water resistance/ Adhesiveness- Refer to table no. 6 e) Microbial load- No colonies were seen on plate. Refer to figure no. 3 f) Temperature stability – The formulation at both room temperature and refrigerator temperature was stable and did not show any separation of colorant, and hence were further subjected to QC tests. Whereas, the lip balm stored in incubator showed separation of components of base and melted.

Figure 2: Shampoo

Figure 3: Microbial load of Lip balm

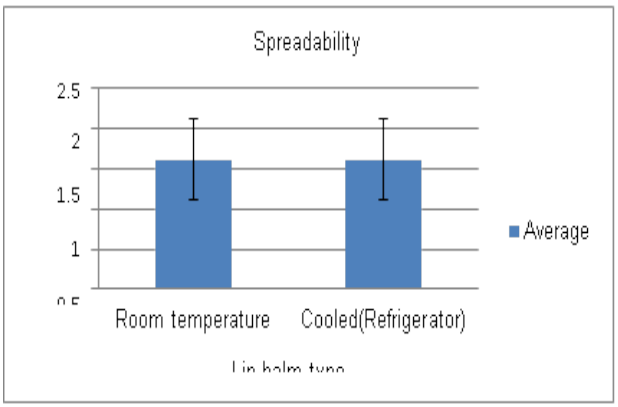

Figure 4: Lip balm spreadability standard error graph

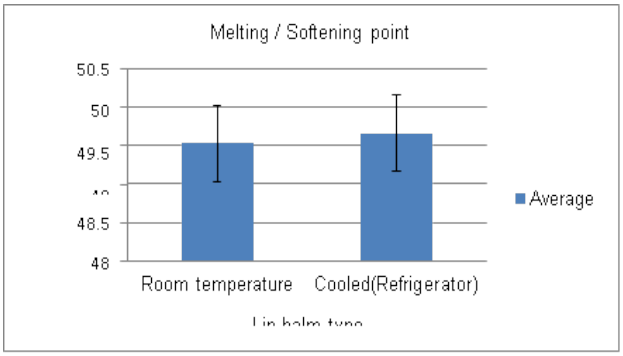

Figure 5: Lip balm Melting point standard error graph

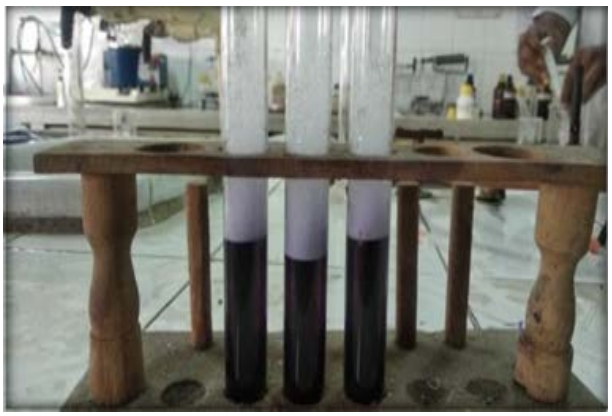

Figure 6: Dirt dispersion of shampoo

Figure 7: Microbial load of shampoo with 0.2% preservative

Figure 8: Microbial load of shampoo with 0.5% preservative

2. Lip balm Standard error bar graphs-
Spreadibility- Refer to table no.7 and figure no. 4 Melting point- Refer to table no. 8 and figure no. 5

3. Shampoo-a) Organoleptic properties- Refer to table no. 9 [17]

b) Foaming and Foam stability- Refer to table no. 10 [17] c) Cleansing action- Refer to table no. 11 [15,17] d) Dirt Dispersion- Refer to figure no. 6 [15,16] Dirt dispersion test of all three samples of shampoo-pH adjusted, non-adjusted and marketed. e) Percentage solid content- Refer to table no. 12 [17] f) Microbial Load- Refer to figure no. 7 for results with 0.2% preservative & figure no. 8 for results with 0.5% preservative. No colonies were found when 0.5% preservative was added. [15] g) Surface tension- Refer to table no. 13 [15,16] h) Bioassay- Refer to table no. 14 and figure no. 9 Was Performed Using three bacteriai. E. coli ii. B. subtilis iii. S. aureus Out of which zone of inhibition was observed only with S. aureus and B. subtilis. No zone of inhibition was observed with E. coli.

4. Shampoo Standard error bar graphs
Foaming and Foam stability- Refer to table no.15 and figure no.10 Solid content- Refer to table no. 16 & 17 and figure no. 11

5. Analysis
a) TLC (Thin Layer Chromatography)- Refer to figure no. 12 Left to right- solvent system no.- 1, 2A and 2B Rf (retention factor) = Ds/Df. It is distance travelled by the compound divided by the distance travelled by the solvent. i. Rf 1= 0.51 ii. Rf 2A= 0.55 iii. Rf 2B= 0.5 b) Uv- Vis- Refer to figure no. 13. Lambda max was observed at – 476 nm. 9 c) Phytochemical Analysis – Refer to table no. 18 and figure no. 14. [11]

6. Solubility – Refer to figure no. 15. The order of solubility of colour concentrate was-Hydro-alcoholic mixture > water > paraffin oil.

7. Colour- Refer to figure no. 16. Best colour was given by pH non-adjusted Hydro-alcoholic solvent. [8,9]

8. Extraction- The colour concentrate obtained from soxhlet was charred and was sticky. But maceration gave proper consistency good colour extract and thus was selected as method of extraction of colorant.

DISCUSSION:

1. Solubility, Colour And Extraction-Hydro-alcoholic extract (non-pH adjusted) solvent was concluded to be a better solvent for extraction and maceration was the method used.2. TLC-Solvent system used showed presence of anthocyanin in colour extract. 3. Uv-Vis Spectra-The lambda max for anthocyanin in hydro alcoholic extract of teak leaf powder was observed at 476nm with an absorbance of 0.61899. 9 4. Phytochemical- Results of few phytochemical test did not match with results in the reference article.11 It was inferred that this might be due to the less percentage of methanol (i.e. 60%) in the used extract as oppose to pure methanol extract used in the reference article. (Refer to table no. 18)

5. Formula a) Lip balm- (Refer to table no. 1)

• The lip balm formulated according to formula 1, showed presence of small lumps and turned out to be a bit sturdy. Also, the amount of colorant added did not give sufficient payoff and showed separation.

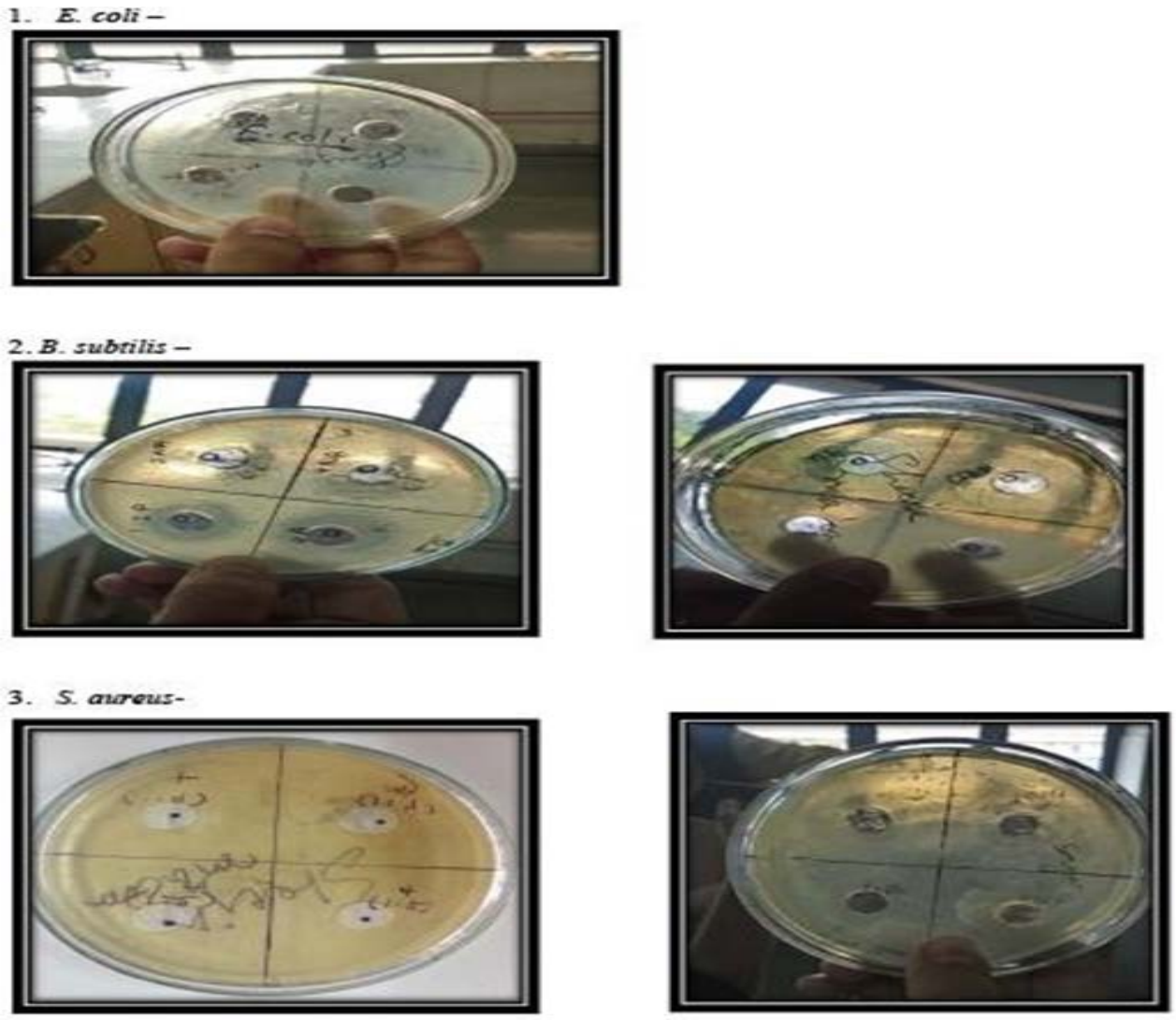

Figure 9: Bioassay of shampoo

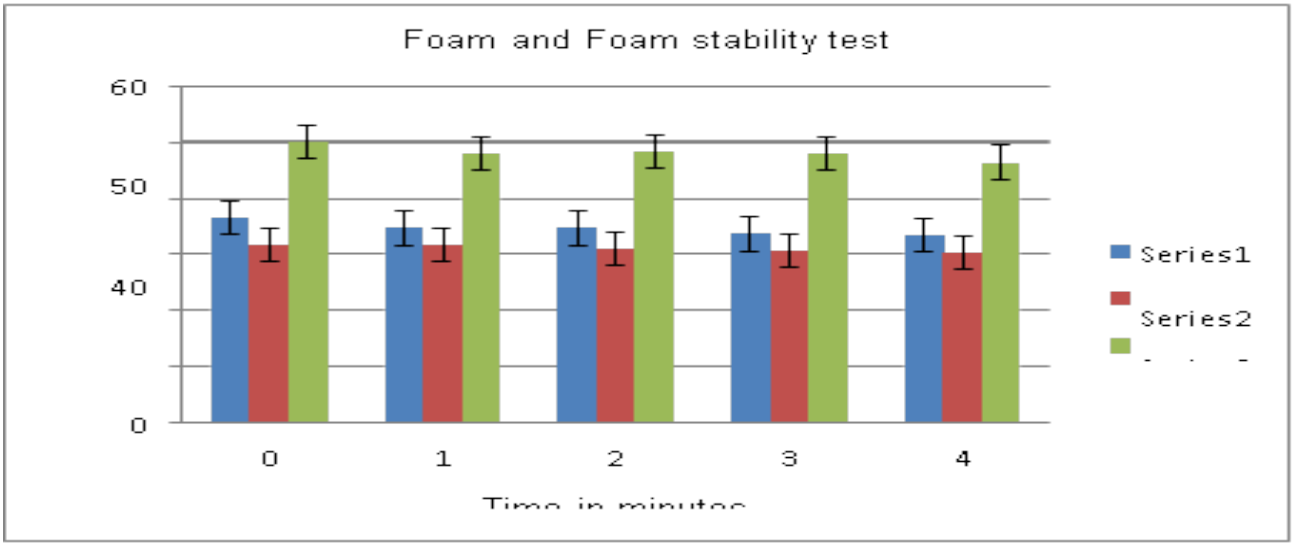

Figure 10: Shampoo foam stability standard error graph

Table 1: Lip balm formula

Ingredients	Formula 1		Formula 2	
Base Quantity(Only) (in g.)	7.5 g	100 g	8.5 g	100 g
Beeswax	1.5 g	20% w/w	1.5g	17.65% w/w
Cocoa butter	1 g	13.33% w/w	1 g	11.7% w/w
Petroleum jelly	1 g	13.33% w/w	1.5 g	17.65% w/w
Liquid paraffin	4 ml	53.33% w/v	4.5 ml	53% w/v
Lemon oil	q.s.	q.s.	q.s.	q.s.
Colorant	1 ml	_	3.5 ml	_

q.s.- Quantity sufficient

Table 2: Shampoo formula

Ingredients	Formula 1 (pH Adjusted)	Formula 2 (pH Adjusted)	Formula 3 (pH Non-Adjusted)	Formula 4 (pH Non-Adjusted)
Sodium lauryl ether sulphate	2.2 ml	2.2 ml	2.2 ml	2.2 ml
Water	2.4 ml	2.4 ml	2.4 ml	2.4 ml
Methyl paraben	0.2% w/v	_	_	_
Sodium benzoate	_	0.2% w/v	0.2% w/v	0.5% w/v
Colorant	1 ml	0.5 ml	0.5 ml	0.5 ml
Perfume	2 drops	2 drops	2 drops	2 drops
Citric acid	q.s.	q.s.	_	_
Total	5.6 ml	5.1ml	5.1ml	5.1ml

q.s.- Quantity sufficient

Table 3: Organoleptic properties of Lip balm

Type	Color	Shine	Odour	Consistency
Room Temperature	Reddish brown	Shiny	Lemon	Smooth
Cooled	Reddish brown	Shiny	Lemon	Smooth

Table 4: Spreadability of Lip balm

Type	D1	D2	D3
Room Temperature	1.6 cm	1.6 cm	1.6 cm
Cooled	1.6 cm	1.6 cm	1.6 cm

Table 5: Softening/ Melting point of Lip balm

Type	Temperature-1	Temperature-2	Temperature-3
Room Temperature	48.6°C	49°C	51°C
Cooled	49°C	49°C	51°C

Table 6: Water resistance/ Adhesiveness of Lip balm

Type	Wt. of empty slides	Wt. after rubbing two slides against each other	total amt. of lip balm on each slide	10% of total amount	#1 difference in wt. of slide after dipping	#2 difference in wt. of slide after dipping
Room Temperature	5.54 g 5.86 g	5.55g 5.90g	0.01g 0.04g	0.009g 0.036g	0.01g 0.0095g	0.03g 0.029g
	5.53g 5.86g	5.55g 5.89g	0.02 g 0.03 g	0.018g 0.027g	0.0082g 0.019g	0.019g 0.029g
Cooled	4.60g 5.55g	4.62g 5.57g	0.02g 0.02g	0.18g 0.18g	0.010g 0.010g	0.020g 0.014g
	5.53g 5.86g	5.55g 5.89g	0.02 g 0.03 g	0.018g 0.027g	9.010g 0.010g	0.015g 0.018g

Wt.- Weight

Table 7: Lip balm Spreadability graph table

Type	D1	D2	D3	Avg.	Std. Dev.
Room Temperature	1.6 cm.	1.6 cm.	1.6 cm.	1.6 cm.	2.719E-16
Cooled	1.6 cm.	1.6 cm.	1.6 cm.	1.6 cm.	2.719E-16

D- Diameter Avg- Average Std. Dev.- Standard deviation

Table 8: Lip Balm Melting point graph table

Type	Mp1	Mp2	Mp3	Avg. in (^{0}C)	Std. Dev.
Room Temperature	48.6^{0}C	49^{0}C	51^{0}C	49.53333333	1.285820101
Cooled	49^{0}C	49^{0}C	51^{0}C	49.66666667	1.154700538

Mp- Melting point Avg- Average Std. Dev.- Standard deviation

Table 9: Organoleptic properties of Shampoo

Type	Color	pH	Odour
Non-Adjusted pH	Orange	5-6	Lemon
Adjusted pH	Orange	3-4	Lemon

Table 10: Foam Stability of Shampoo

Type	0 Minute	1 Minute	2 Minute	3 Minute	4 Minute
Adjusted pH	36.66 ml	34.66 ml	34.66 ml	33.66 ml	33.33 ml
Non Adjusted pH	31.66 ml	31.66 ml	31 ml	30.66 ml	30.33 ml
Marketed	50 ml	48 ml	48 ml	48 ml	46.33 ml

Table 11: Cleansing action of Shampoo

Type	Wool (In Grams)	Wt. of Oil + Wool	Wt. after drying	Cleansing action in percentage
Adjusted pH	5.13 g	8.92 g	7.3 g	44 %
Non Adjusted pH	5.2 g	8.1 g	7.26 g	41 %
Marketed	5.0 g	8.04 g	7.73 g	55 %

Table 12: Percentage solid content of Shampoo

Type	Percentage Solid Content
Non Adjusted pH	16.42%
Adjusted pH	17.42%
Marketed	17.83%

Table 13: Surface tension of Shampoo

Type	Surface tension
Non adjusted pH	29.4268 N/m
Marketed	79.1775 N/m

Table 14: Bioassay of Shampoo

Type	Zone Of Inhibition (in cm.)	
Bacteria	*B. subtilis*	*S. aureus*
Adjusted pH(1:2)	2.1	1.5
Adjusted pH(1:4)	2.1	1.5
Non-Adjusted pH(1:2)	2.2	1.6
Non-Adjusted pH(1:4)	2.7	1.7
Marketed(1:2)	3	2.6
Marketed(1:4)	3.2	2.5

1:2 – 1 part of formulation diluted with 2 parts of water
1:4 – 1 part of formulation diluted with 4 parts of water

Table 15: Shampoo foam stability graph table

Minute	Type	R1 (in ml.)	R2 (in ml.)	R3 (in ml.)	Avg.(in ml.)	Std. dev.
0	Adjusted pH	36	38	36	36.66666667	1.154700538
	Non-Adjusted pH	29	34	32	31.66666667	2.516611478
	Marketed	50	50	50	50	0
1	Adjusted pH	34	36	34	34.6666667	1.154700538
	Non-Adjusted pH	29	34	32	31.6666667	2.516611478
	Marketed	48	48	48	48	0
2	Adjusted pH	34	36	34	34.6666667	1.154700538
	Non-Adjusted pH	29	33	31	31	2
	Marketed	49	48	48	48.3333333	0.577350269
3	Adjusted pH	34	34	33	33.6666667	0.577350269
	Non-Adjusted pH	28	33	31	30.6666667	2.516611478
	Marketed	48	48	48	48	0
4	Adjusted pH	33	34	33	33.3333333	0.577350269
	Non-Adjusted pH	28	32	31	30.3333333	2.081665999
	Marketed	46	46	47	46.3333333	0.577350269

R- Reading Avg- Average Std. Dev.- Standard deviation

Table 16: Shampoo solid content graph table 1

Type	R. No.	Wt. Of Empty Evaporating Dish (in g.)	Wt. After 4g Of Shampoo (in g.)	Wt. After Drying (in g.)	Difference After Drying (in g.)	Solid Content After Drying (in g.)
Non- Adjusted pH	1	60.66	64.66	60.98	3.68	0.32
	2	63.62	67.62	64.41	3.21	0.79
	3	72.84	76.84	73.7	3.14	0.86
						Avg. = 0.656666667
						Std. Dev. = 0.293655127
Adjusted pH	1	76.62	80.62	77.4	3.22	0.78
	2	65.16	69.16	65.86	3.3	0.7
	3	76.62	80.62	77.23	3.39	0.61
						Avg. = 0.696666667
						Std. Dev. = 0.085049005
Marketed	1	67.71	71.71	68.52	3.19	0.81
	2	63.59	67.59	64.41	3.18	0.82
	3	75.87	79.87	76.38	3.49	0.51
						Avg. = 0.713333333
						Std. Dev. = 0.176162803

Wt.- weight Avg- Average Std. Dev.- Standard deviation

Table 17: Shampoo solid content graph table 2

Type	Solid Content (Avg.)	Std. Dev.	Percentage Solid Content
Non-Adjusted pH	0.656666667 g	0.293655127	16.42%
Adjusted pH	0.696666667 g	0.085049005	17.42%
Marketed	0.713333333 g	0.176162803	17.83%

Avg- Average Std. Dev.- Standard deviation

Table 18: Phytochemical analysis

Tests	Results
1. Steroids	+++
2. Tannins	+++
3. Saponins	+++
4. Coumarin	-
5. Emodins	++
6. Carbohydrates (Iodine test)	-
7. Diterpenes	+++
8. Cardiac glycosides (Keller Killiani)	-
9. Chalcones	+
10. Phenol	++
11. Phlobatannins	-
12. Proteins	-
13. Phytosterol	++
14. Flavonoids	-
15. Anthocyanin	+++
16. Alkaloids	-
17. Leucoanthocyanin	-

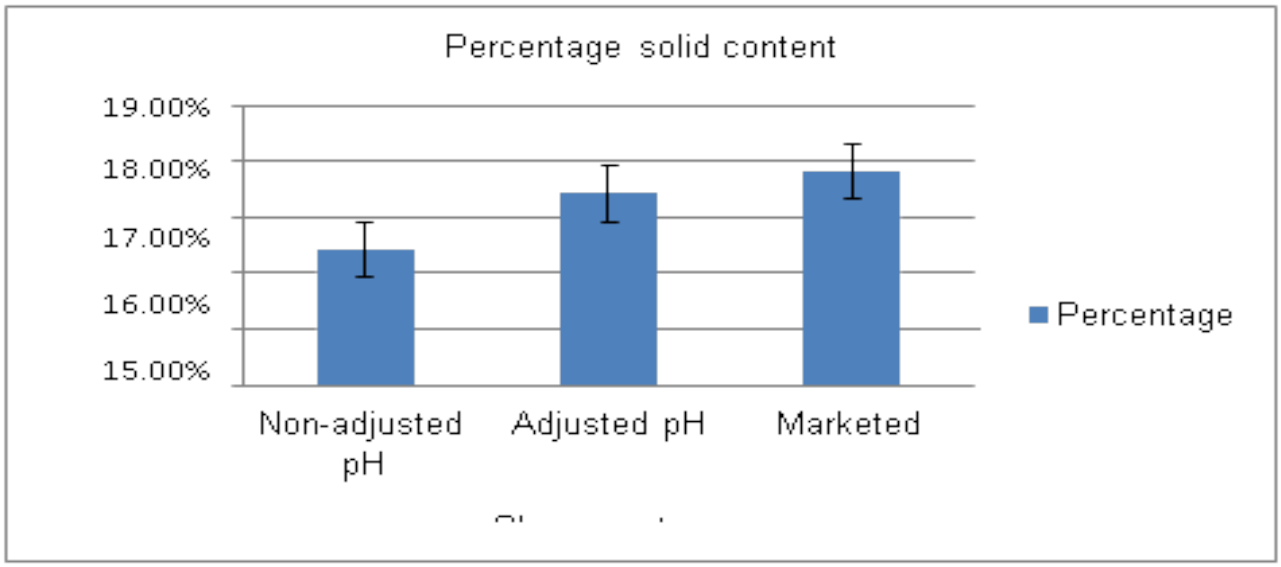

Figure 11: Percentage solid content standard error graph of shampoo

• So, in the lip balm formulated according to formula 2, method A the quantity of liquid paraffin and petroleum jelly was increased to tackle the problem of sturdiness and lumps, and amount of colorant was also increased.

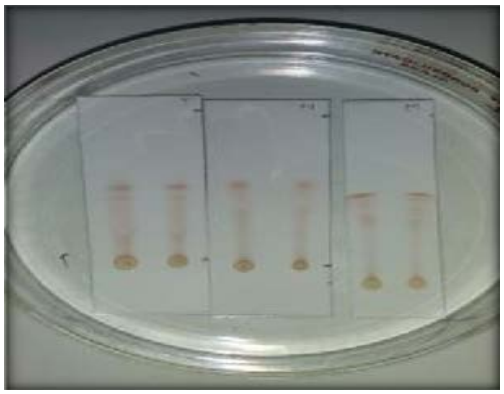

Figure 12: TLC Plates

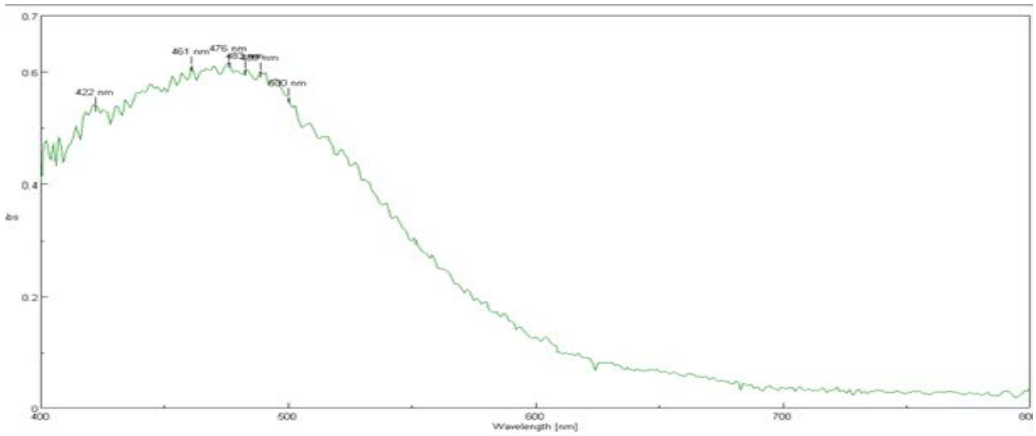

Figure 13: Uv-vis spectra

- Lip balm formulated by formula 2, method A, was free from lumps but showed colour separation.
- So, lip balm was then made by using formula 2, method B, in the set base was triturated with the concentrated colorant as opposed to method A where the colorant 18 was directly added into the molten base. This showed no colour separation and no lump formation.
- Lip balm formulated by formula 2, method B was stored at room temperature, in incubator (temperature slightly higher than room temperature- 370C - 400C) and in refrigerator to check for stability. b) Shampoo- (Refer to table no. 2)
- In formula 1, methyl paraben was used as a preservative, but since it requires heat to get solubilized into the water, it created turbidity during formulation of shampoo.
- In formula 2, to tackle with problem of methyl paraben, sodium benzoate was used as a preservative since it is soluble in water and does not need application of heat for the same. Also, amount of colorant in formula 2 was reduced for aesthetic purpose, and was kept constant for the remaining formulas.

Figure 14: Phytochemical test results

• Formula 2 & 3 were compared for stability and appearance of colour in shampoo. The pH was adjusted in formula 2 to 3-4 using citric acid since the colorant used shows maximum stability at that pH.

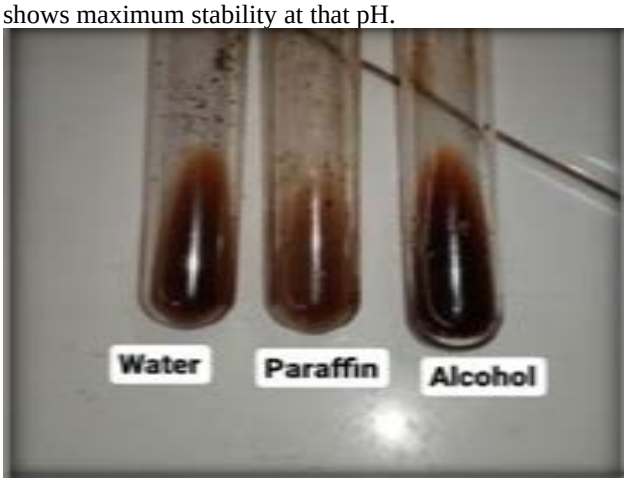

Figure 15: Solubility of extract

• Formula 3 was formulated in similar manner to formula 2 except that it was not adjusted for pH.

• Formula 3 formulated shampoo, was tested for microbial load test and it showed microbial growth.

Figure 16: Colour test of the extract.

• Thus, in formula 4, amount of preservative was increased; and finally, formula 2 & 4 was finally tested for all Quality control tests, to compare between pH adjusted and non-adjusted formulation, except for microbial load; and the observed results were similar for both formulation but since formula 4 had correct amount of preservative, it did not show any microbial growth and was thus finalized. [19]

6. Evaluation tests-

a) Lip Balm-

• The organoleptic properties and spreadability remained same for both lip balms.12 (Table no. 3 & 4)

• Also melting poitns were observed at similar range. 12 (Table no. 5)

• Results of water resistance test, showed around 10% loss of lip balm after second water dip. (Table no. 6)

• No microbial load was observed. (Figure no. 3)

• Stability test- Room temperature and refrigerator sample was stable.

b) Shampoo-

• Organoleptic properties of both pH adjusted and non-adjusted formulation as per formula 2 & 4 respectively remained the same. [17] (Table no. 9)

• Cleansing action of marketed shampoo was better than test formulations, because of greater amount of surfactant in it.[17] (Table no.11)

• Foam stability observed in descending order as follows – (Table no. 10) (pH nonadjusted formulation > pH adjusted formulation > marketed formulation.)[17]

• All three samples of shampoo- adjusted, non-adjusted and marketed passed the test dirt dispersion test and showed similar results.[15,17] (Figure no. 6)

• If shampoo has too many solid content, it is difficult to wash out, the test formulations have solid content between 16- 18% which is similar to the marketed formulation.[15,17] (Table no. 12)

• In Bioassay, for all three formulations, zone of inhibition was observed only with S. aureus and B. subtilis. No zone of inhibition was observed with E. coli. Since zone of inhibition was observed for two out of three bacteria for all three formulations, the preservative used was effective. [18] (Table no. 14 & Figure no. 9) [20]

• Formula 3 & 4 were tested for microbial load, out of which formula 3 formulation showed microbial growth and formulation by formula 4 did not. (Figure no. 7 & 8)

• Surface tension is reduced but results do not match with marketed formulation.[15] (Table no. 13)

CONCLUSION

Natural colorants are proven safe and effective for use in cosmetic formulation. The study in this article is premised on the above statement which involves formulating cosmetics using a natural colorant. A better form and shade of the colorant from teak leaves, was obtained without the need for adjusting pH of the solvent by using maceration as opposed to soxhlet. The presence of anthocyanin, the colouring pigment was detected successfully in the hydroalcoholic extract, by using Thin layer chromatography and UV- vis spectra whereas phytochemical analysis failed to do so. Formula 2 lip balm, formulated using method B was finalized; since it gave a homogenous formulation with a good pay off, showed temperature stability, melting point around 500C and satisfactory results for other QC tests. The concentrated extract of colorant was incorporated in lip balm by trituration. Shampoo comprised of hydro-alcoholic extract of colorant and best shampoo was obtained by using formula 4. This formula did not show any microbial growth and gave a stable product and thus was finalized. So, the teak leaf extract gave a good colour, it did not separate out or showed any blooming or fading throughout the stability studies. The positive results indicate that, the teak leaf extract can be used as a colorant to formulate both aqueous base and lipid based cosmetic.

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