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Original Article

DEVELOPMENT OF COSMECEUTICAL PRODUCTS FROM NANO-SIZED ACTIVE COLOUR CONSTITUENTS OF RATANJOT, SEABUCKTHORN AND ANNATTO

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ABSTRACT

Objective: The objective of this research was to develop the nano-particles of natural colours from Ratanjot root, Seabuckthorn pulp, and Annatto seeds and use them in cosmetic products. Multiple herbs were used to have a good mix of active colour constituents and have a possibility to create a range of shades in the final cosmetic formulation without any side-effects. Further, with nano-sizing of the colour constituents, we aimed to obtain better activity and enhanced performance in the final product.

Methods: The extraction of natural colours from herbal source was carried out under controlled conditions of temperature (max. 50 °C) and pressure (50-60 mm of Hg) to minimize the loss of actives during the process. The colour fractions separated by solvent fractionation were nano-sized by ultra-sonication using polyethylene glycol 400 as additive to distribute uniformly in the formulation. Active colour components were analyzed by HPLC and Lovibond Tintometer to maintain the colour strength in cosmetic formulation.

Results: The nano-sizing of natural colour (purplish red dye from Ratanjot root, oil of Seabuckthorn pulp) has resulted in giving an excellent gloss, spreadability and prevention of skin damage due to their good anti-oxidant property. The goodness of Seabuckthorn oil has rendered a special effect of rejuvenating the skin cells to retain their elasticity.

Conclusion: The promising results of these formulations have encouraged us to go for pilot trials to extend the study on animal and human subjects for the skin-penetration and kinetics of their healing power.

Keywords: *Bixa orellana*, *Arnebia nobilis*, *Hippophae rhamnoides*, Herbal lipstick, Herbal lip balm, Body Massage oil, Cosmetics, Carotenoids

INTRODUCTION

These days, the usage of herbal cosmetics has increased many folds in personal care systems [1]. In the case of colour-based cosmetics like lip-care, foundation, etc., now people want to use natural colouring material instead of synthetic colours. In addition, they are also looking out for other effects like UV protection, anti-aging, even-toning, etc. to just the colourful shades and gloss. It has been found that there are natural colouring agents that can double up as a colour source and as a bioactive phytochemical. Using such natural colouring agents not only improves or maintain the status of skin without any side effects but also greatly helps men and women to look more impressive, beautiful and smart to a considerable extent [1]. These cosmetics provide health benefits that go far beyond the colouring properties alone.

Colouring lips have been practiced since ancient times. In present days, the use of lipsticks has increased, and choices of shades, colour, texture, and lustre have been changed and have become wider [2]. The synthetic colouring dyes used in lipstick are dangerous on consumption in a mild form and can have effects like allergy, drying of lips, nausea, dermatitis and can be carcinogenic. Herbal colours are nontoxic with antimicrobial, antioxidant and anti-inflammatory properties. These colours when added in different combinations, further shades can be obtained. Colours can be changed to different shades with organic and inorganic acid and bases [3].

Lip balm should not be considered equivalent to lip gloss with former being a product intended to use by both men and women. Herbal lip balms are formulated in an effort to produce a safe product for lip care. Herbal formulations of massage oils are being made to produce non-toxic and safe oil that can tone the muscles, heal the wounds, moisturize, sedate the skin that is sensitive to environmental hazards, strengthen the texture of skin and prevent premature aging.

In this work, an attempt has been made to formulate lipsticks, lip balms and massage oil using the natural bio-active phytochemicals/colouring-agents extracted from Ratanjot, Annatto and Seabuckthorn, In the remaining part of this section, we give a brief description of these herbs.

A rich source of natural red colourants based on naphthoquinone molecule is *Arnebia nobilis* Rech. f, commonly known as Ratanjot in India. The name Ratanjot is attributed to roots of the various Boraginaceous plant species. It is not only an important herbal drug of the indigenous system of medicine but also used to impart pleasing red colour to food oils and fats. Naturally occurring hydroxynaphthoquinones especially isohexenylnaphazarins commonly known as shikonin and alkanin are lipophilic red pigments which are known to have been isolated from the roots of various species of family boraginacea [4]. They form the main active constituents of this plant and also are responsible for its colour and therapeutic efficacy. They are reported to have antimicrobial, anticancerous, antitumor and wound healing properties [5, 6].

Bixa orellana Linn family (bixacae) commonly known as Annatto, is an evergreen shrub which grows up to 5-6 meters with peculiar reddish fruits enclosing 10-15 seeds. Bixin, a carotenoid also known as a seed specific pigment widely used in food and cosmetic applications, antioxidant properties of which are well-known [7]. *Annatto* extract is particularly useful because it is available in both water soluble and oil soluble form to produce orange, yellow and red hues [8].

Hippophae rhamnoides L. family (Elaeagnaceae) commonly known as Seabuckthorn, which grows widely in cold regions of Indian Himalayas, China, Russia, Europe and many countries has recently attracted the attention of the scientific community. The fruits and leaves of this plant are quite rich in anti-oxidants like vitamin C, E, carotenoids, flavonoids and sterols which have found application in the preparation of health protection food, drug, and cosmetic products. Sea buckthorn has been shown to have potent antioxidant activity [9, 10]. Seabuckthorn oil has a UV blocking property as well as emollient properties and it is an aid in promoting regeneration of tissues [11].

The rationale behind using these three herbs in preparing cosmetic formulations was two-fold. Firstly, with all the three herbs known to be rich in colour, it facilitated access to multiple natural colours which we could use to generate a range of shades in the cosmetic formulations without introducing any side-effects. Secondly, with Seabuckthorn and Annatto being rich in anti-oxidants, and with Ratanjot having anti-microbial and anti-tumor properties, we aimed to provide a health benefit of a broad-spectrum nature in the final formulation. Earlier works in natural cosmetics have focussed on using only a single herb like Annatto [12] or using common herbs like beetroot[3,13-15], turmeric[16], usual fruits[17] as the colouring agent. Further, we have adapted the phytochemical extraction processes to obtain the extract in its natural form and nano-sizing has been performed to enhance the spread, hue and colour in the final formulation, which also adds to the novelty of this work.

MATERIALS AND METHODS

Collection of plant material

The various herbs used in the formulation of herbal lipsticks were selected on the basis of literature survey [4, 18, 19]. The Annatto seeds were gifted by Indo Nacop Chemicals Ltd. Vinukonda (Andhra Pradesh, India). This company collects the seeds through the agency for the Eastern Ghats spread over a large area from Baster (Chhattisgarh) to Vishakhapatnam. The Seabuckthorn berries pulp was procured from Leh through IIT Delhi (India) in frozen condition. The Ratanjot root was purchased from the local herbal market, khari baoli, Delhi, India.

Extraction of plant material

I. Seabuckthorn pulp extraction process

The extraction of Seabuckthorn pulp oil has been done earlier, but by adopting a different route. In [20, 21], oil was separated from pulp juice by high-speed centrifugation, removing the oil by density difference but complete extraction was not achieved. In [22], the oil was separated by refluxing with ethyl alcohol following by purification with n-hexane. The process that we have adopted and described below is carried out at low temperature and pressure conditions to prevent the loss of antioxidant activity of the oil.

500 g pulp with moisture contents of 83.57% (Karl Fischer method) was transferred in a 5 l glass reactor fitted with a stirrer (90 rpm) and 0.5 l ethyl alcohol was added with stirring. The contents were stirred for 1 h and filtered on 300 mesh cloth. The pulp left on the cloth was again stirred with 250 ml ethyl alcohol for 30 min and filtered on 300 mesh cloth. The alcohol insoluble pulp obtained was reddish-yellow in colour and alcohol soluble fraction was pale yellow in colour. The alcohol was distilled off leaving behind 45 g concentrate composed mainly of carbohydrates and polar compounds (vitamin C, B-complex, etc.). The pulp was then treated with 100 ml acetone and 100 ml n-hexane in a high shear mixer at room temperature for 2 h. It was then filtered under vacuum. The residue on filter paper was washed with hexane. The filtrate and washings were collected, and the solvent was distilled off under vacuum. This gave a dark yellowish red oil (yield = 18 g). The fibre after drying was obtained with a yield of 11.85 g. This process has been evolved by conducting series of experiments to extract oil & vitamin rich concentrate in its natural form. The fibre was obtained as a by-product. The obtained oil was analysed for colour units:

Red unit (Lovibond Tintometer, Model F, Visual)	5 (1.0% solution in sesame oil)
Yellow unit (Lovibond Tintometer, Model F, Visual)	22 (1.0% solution in sesame oil)

Table 1: Analysis of the seabuckthorn oil

Property	Value
Refractive Index	1.47
Acid value	7.6
Specific gravity	0.82
Sterol (B-sitosterol)	0.56% *(by HPLC method)*
Total tocopherols	1.1% *(by HPLC method)*
Total carotenoids	0.52% *(by HPLC method)*

Nano-sizing of seabuckthorn oil

The nano-sizing of the oil was done to have an enhanced spread of the formulation with excellent colour & hue.

Methods

Seabuckthorn oil was solubilized in polyethylene glycol 400, Tween 80 and poloxamer which belongs to GRAS category. A ratio of 1:3 mixture of PEG 400 and water was prepared and sonicated using handheld ultrasonicator for 15 min at an amplitude of 1.0. Thereafter, the sample was sent for size analysis using Malvern zeta sizer. The detailed report is shown in fig. 1.

Results

The average particle size was found to be 142.4 nm.

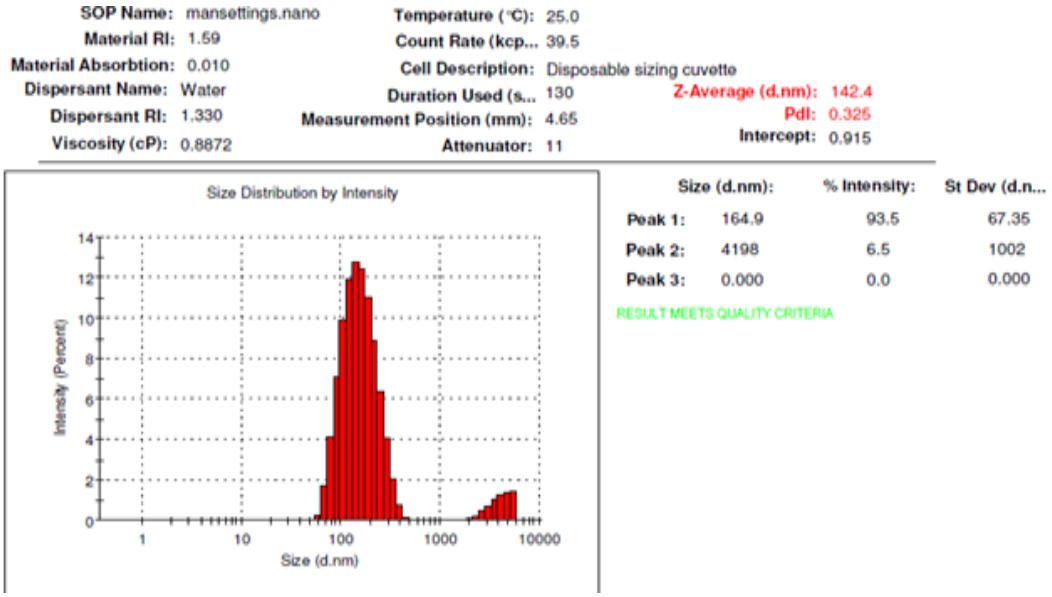

Fig. 1: Particle size distribution histogram (Seabuckthorn oil)

II. Ratanjot root extraction process

There has been some work reported [4, 23] on extraction and isolation of dyes from Ratanjot, but in this work, extraction of colour fractions has been done at low temperature and pressure conditions followed by nano-sizing, as described further. 500 g of Ratanjot root was crushed to coarse powder about 3 mm to 6 mm size and extracted in soxhlet using hexane as the solvent for 8 h. Most of the oil-soluble dye got extracted in the hexane. The dye was recovered from oil soluble hexane by distilling of hexane under vacuum at a temperature, not more than 50 °C. The purplish red dye thus obtained was suspended in about 10 times the quantity of dye in refined castor oil and was homogenized at 10000 rpm and then in a high-pressure homogenizer at 1000 bar. This gives a crystal clear solution with particle size 0.5 µm to 3 µm. The dye was analyzed for red and blue units.

Red unit (Lovibond Tintometer)	12 (0.5 % solution in sesame oil)
Blue unit (Lovibond Tintometer)	8 (0.5 % solution in sesame oil)

Nano-sizing of ratan jot

The idea behind achieving nano-sizing is that the nano-sized formulation containing this constituent will have enhanced spread and colour.

Methods

Ratanjot was solubilized in polyethylene glycol 400 which belongs to the GRAS category. A ratio of 1:3 mixture of PEG 400 and water were prepared and sonicated using handheld ultrasonicator for 15 min at an amplitude of 1.0. Thereafter, the sample was sent for size analysis using Malvern zeta sizer. The detailed report is shown in fig. 2.

Results: The average particle size was found to be 199.3 nm.

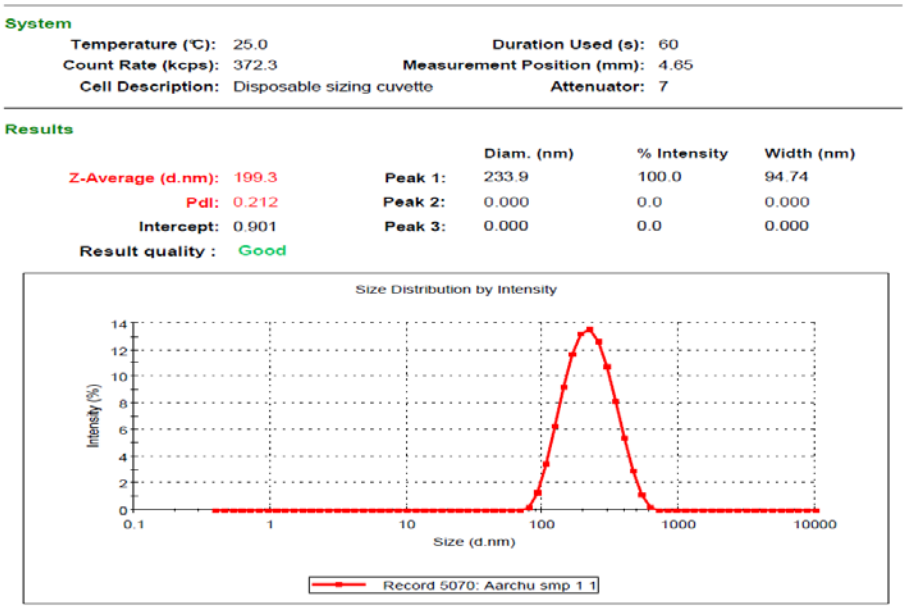

Fig. 2: Particle size distribution histogram (Ratanjot)

III. Annatto seeds extraction process

The extraction of bixin and oil-soluble annatto extract has already been done earlier [24-26], but process shown here is carried out by optimizing the duration of extraction and temperature and separation techniques to have a good recovery of actives.

5 kg seeds of 2.5% bixin contents were extracted with ethyl acetate in pilot plant fitted with circulation pump and stirrer. The seeds were first loaded into the extractor, and the 15 l ethyl acetate was charged through the pump. The solvent was circulated and simultaneously the temperature was raised to 50 °C. The extraction was continued for 2 h and then the micelle (extracted product) was transferred to the distillation unit for recovering ethyl acetate; the seeds left were extracted two more times the same way as above. The micelle was transferred to the same distillation unit. The recovery of ethyl acetate was carried out till final volume reaches to 10 l. It was collected in a stainless steel container and kept at 10 °C overnight. The crystals of bixin were separated out which were filtered through vacuum filter and dried. The filtrate was collected and transferred to a distillation unit. The ethyl acetate was recovered completely, and left product was in paste form. The product (350 g) was digested in the olive oil/sesame oil three times at 50 °C for 1 h. The product obtained was micro-sized in Ultra-sonicator (time 10 min amplitude 1.0). The particle size was analysed by master size (Malvern) and 70% particles were within the range of 500 nm. Since the particle size was already in the desired range, we decided not to carry out further sub-sizing. The obtained bixin was analysed for following colour units:

Red unit (Lovibond Tintometer)	18
Yellow unit (Lovibond Tintometer)	32

The Bixin content was found to be 0.45% by HPLC (equipment and conditions are shown in table 2) Bixin peak appeared at 8.06 min. The final oil soluble dye was preserved in an amber coloured bottle kept in dark at ambient temperature.

Fig. 3: Prepared natural lipsticks

Table 2: HPLC equipment and conditions for Bixin content analysis

Equipment/Condition(s)	Value(s)		
Instrument, Column	Agilent infinity 1220, Eclipse plus C-18 (4.6x250 mm)		
Mobile phase	0.1% acetic acid (A) acetonitrile(B)		
Run-time, Flow-rate, Wavelength	20 min., 1 ml/min., 470 nm		
Column Temperature	45 °C		
Gradient	Time	A	B
	0.0	25	75
	11.0	05	95
	18.0	05	95

Preparation of formulations

The bio-actives extracted from the plant materials were used in preparing natural lipsticks, lip balm, and body massage oil. They were formulated as per method described [27-29]. The ingredients used in the formulation of lipstick, body massage oil and lip balm are shown in table 3, 4 and 5 respectively.

I. Formulation of natural lipstick

Three variants (NL-1, NL-2 and NL-3) of the natural lipstick were formulated by carefully varying the contents of Ratanjot pigment, Annatto dye and Seabuckthorn oil to obtain different shades (see fig. 3). The variation of constituents in the three lipsticks can be seen in table 3 (rows 8, 9 and 10)

Table 3: Lipstick using ratan jot, annatto and sea buckthorn extracts

S. No.	Raw materials	% in NL			Function
1	Candelia wax	4.00			Binding agent, water repellent property
2.	Carnuba, Ozokerite, Bee wax	1.80, 7.00, 2.50			Glossy and hardness
3.	Microcrystalline wax	7.00			Binder, emulsion stabilizer, viscosity increasing agent
4.	Shea butter	2.00			Emollient
5.	Light liquid paraffin	60.4			Blending base, lubricating agent, resist moisture
6.	Caprilic acid triglycerides	5.00			Emulsifier
7.	Methyl paraben, Propyl paraben	0.20, 0.10			Preservative
		NL-1 (Maroon)	NL-2 (Red)	NL-3 (Purple)	
8.	Ratanjot pigment	4.0	3.0	5.0	Antioxidant and Colouring agent
9.	Annatto dye	3.0	3.0	4.0	Antioxidant and Colouring agent
10.	Seabuckthorn oil	3.0	4.0	1.0	Antioxidant and Colouring agent

Table 4: Preparation of body massage oil from annatto and seabuckthorn oil

S. No.	Particulars	%	Function
1	Turmeric oil	0.5	Anti-inflammatory
2	Annatto oil	1.0	Antioxidant
3	Sesame oil	78.3	Antibacterial, anti-aging, moisturizer, emollient
4	Malkangani oil	5.0	Nervous stimulant
5	Sesame oil ester	15	Lubricant
6	Tocopherols from Soy	0.1	Antioxidant
7	Seabuckthorn oil	0.1	Antioxidant

Table 5: Preparation of lip balm from annatto seeds and ratanjot root extracts

S. No.	Phase	Particulars	%	Function
1	A1	Paraffin wax	15	Binding agent, blending base
2		Candellila wax	0.2	Binding agent, water repellent property
3		Micro crystalline wax	15	Binder, emulsion stabilizer, viscosity increasing agent
4		Cetyl Palmitate	2.5	Emulsifier, emollient, occlusive and masking agent
5		Light liquid paraffin	55.4	Blending base, lubricating agent, resist moisture
6		Butylated hydroxyl Toluene	0.1	Antioxidant, anti rancidifier
7		Shea butter	1.0	Moisturizer, skin-rejuvenator
		Phase A1 (Sub Total)	89.2	
1	A2	Wheat germ oil	0.1	Antioxidant, anti-aging property
2		Betel leaf oil	0.1	Emollient, Flavouring agent
3		Oryzanol, Tocopherols from Soy	0.1, 0.1	Anti-oxidant
		Phase A2 (Sub Total)	0.4	
1	A3	Annatto extract(Colour oil soluble)	0.1	Antioxidant and Colouring agent
2		Ratanjot extract (Colour oil soluble)	0.1	Antioxidant and Colouring agent
3		Sesame oil	9.9	Antibacterial, anti-aging, moisturizer, emollient
		Phase A3 (Sub Total)	10.1	
1	A4	Flavours Strawberry 66061 Ultra Sahibabad	0.3	Flavouring agent
		Phase A4 (Sub Total)	0.3	
		Total	100.00	

II. Formulation of natural body massage oil

Body massage oil was prepared using all the natural ingredients with Sesame oil as the base. Annatto and Seabuckthorn oil were added to provide antioxidant and anti-aging properties to the oil. The detailed composition is shown in table 4.

III. Formulation of natural lip balm

Multiple formulations of natural lip balm were prepared, and the best one has been reported with the composition described in table 5. Natural colour fractions from annatto and ratan jot were added to provide both colour and antioxidant/antimicrobial property to the formulation. Few other antioxidants were added to obtain a broad-spectrum activity. We prepared and added betel leaf oil as a flavouring agent. Betel leaf [30] oil was extracted by dehydrating the betel leaves on a zeolite bed under high vacuum. The obtained leaf powder was then macerated with olive oil (1:20) for 12 h to get clear oil with the aroma intact. The betel oil aroma was blended with the strawberry flavour with an aim to render a subtle sweet and pleasing odour to the lip-balm.

RESULTS AND DISCUSSION

It is essential to maintain a uniform standard for herbal lipstick, keeping this view in mind the formulated natural lipstick was evaluated on the parameters such as melting point, breaking point, the force of application, surface anomalies, etc. [27]. On similar lines, the formulated lip balm and the body massage oil were also evaluated. This evaluation was done using well-known standard methods. The inferences drawn from the evaluation are shown in table 6. The lipstick and lip balm formulations developed showed appropriate organoleptic characteristics and were solidified without deformation. The stability was considered good for formulations stored at room temperature and under refrigerated conditions. Suitable combinations of plant extracts with other ingredients in formulations provided different shades of lipsticks and lip balms. Body massage oil showed excellent properties like stability, spreadability, non-stickiness and its suitability for tender to aged skin. The nano-sizing of natural pigments has provided good spread, gloss and colour stability in all the formulated products.

In the past few years, people have noticed many side-effects to the severity of skin cancer, due to the presence of artificial chemicals in the cosmetics. So, the need of the hour is to shift towards natural ingredients in cosmetic products to not only ward off the side-effects but also reap in a range of benefits like improved skin health, anti-aging, etc. In the last couple of years, there has been some work in this direction [3, 12-17], but the scope was limited to using common natural ingredients like beetroot, turmeric, common fruit extracts, etc. in the formulation. In this work, we have extended the scope by incorporating ingredients like Ratanjot and Seabuckthorn that haven't been explored much in cosmetics esp. in lip-care. We have also used Annatto that provided us with one more natural colouring agent and helped us in creating more shades of lipsticks and lip balms. Using the actives obtained from these three herbs, we have also imparted a broader health benefit to the final formulation with multiple antioxidants coming from Seabuckthorn and Annatto, while an anti-microbial nature from Ratanjot. In addition, we have adapted the extraction processes of the natural colours to work under controlled conditions of temperature and pressure to minimize the loss of actives. Further, we have performed nano-sizing and used the nano-sized colour particles to prepare the cosmetic formulations that showed enhanced performance when compared to their micro-sized counterparts. With the evaluation showing promising results and coupled with all aforementioned benefits, we have moved ahead to do clinical trials for further studies.

Table 6: Evaluation of natural lipstick, lip balm and body massage oil

Parameter	Inferences		
	Lipsticks	**Lip-balm**	**Body massage oil**
Colour	Maroon, Red, Purple	Light-pink	Light red
pH	5.5 to 7.2	6.2	Neutral
Melting point	55-70 °C	-	-
Coverage[3]	Good	Good	-
Perfume stability[4]	++	++	++
Aging stability[4]	Smooth	Smooth	Smooth
Ease/Force of application[5]	Good	Good	Good
Shrinkage and Grittiness[4]	Smooth and shrinkless	Smooth and shrinkless	-
Solubility	-	Not soluble in water and stable at 4.5 to 7.5 pH	Insoluble in water, washable with soap
Spreadability[3]	Smooth	Smooth	Smooth
Stickiness	-	-	Non-sticky
Surface Anomalies[5]	No defect	No defect	No defect
Skin irritation[6]	No	No	Suitable to tender to aged skin

[1]Measured using pH meter, [2]Determined using capillary tube method, [3]Coverage refers to the opacity provided by the cosmetic formulation; while spreadability corresponds to the drag/friction offered by the formulation on application/removal, [4]Formulation was kept for a stipulated period and properties like fragrance, bleeding, crystallization on surface, shrinkage, etc. were recorded, [5]Force of Application test for lipsticks and surface anomalies test was done as described in [27], [6]Skin irritation was checked by applying the formulation for 10 min over the skin.

CONCLUSION

This research provides a guideline on the use of carotenoid/naphthoquinone based plant products to produce a safe, attractive product with multifunctional usage for skin and lip care formulations. The natural ingredients like Annatto seed extract, Ratanjot root extract, and Seabuckthorn pulp extract, were used in the preparation of lipsticks, lip balms and body massage oil. The promising results of these formulations have encouraged us to go for pilot trials to extend the study on animal and human subjects for the skin-penetration and kinetics of their healing power.

CONFLICT OF INTERESTS

Declared none

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