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ORIGINAL ARTICLE **OPEN ACCESS**

Extraction of Bacterial Pigments and Its Application in Cosmetics

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ABSTRACT

The demand for various cosmetics is on rise and ever increasing due to inherent desire to look beautiful. Recent few years witnessed a marked increase in use of different cosmetic formulations by women all over the world. Varieties of cosmetics from different manufacturers are available in the market. Synthetic colours have been used in cosmetics universally from long ago, resulting in human health risks and environmental pollution. Therefore, it is critical to search novel natural pigments that are safe and alternative to synthetic ones. Compared to synthetic colours, microbial pigments shows better biodegradability and greater compatibility with environment. Thus, in current investigation an attempt has been made to formulate and evaluate various cosmetic formulations namely Lipstick, Rouge and Eye shadow using natural-colored pigments extracted from bacteria to overcome the drawbacks associated with synthetic colorants, since the synthetic colorants might cause allergy and are reported to be carcinogenic. The formulated cosmetics were evaluated for different evaluation tests like colour, texture, melting point etc. depending upon its type. The results of current study verified that the pigments extracted from bacteria may prove to be a safe choice and work as an alternative for the synthetic colorants in various cosmetic formulations.

Keywords: *Bacterial pigment, Lipstick, Rouge, Eye shadow, Cosmetics.*

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INTRODUCTION

Recently demand for cosmetic and personal care products is witnessing substantial growth, due to increased income level, changing lifestyle and urbanization. New generation is experiencing changing standard of living, which creates a vast demand for cosmetics. Increasing financial independence of women has resulted in increased inclination towards colour cosmetic products. Change in lifestyle of rural population together with impact of western culture is also boosting growth of the cosmetics market globally. The size of India's cosmetic market is expected to reach $20 billion by 2025 and India will be the largest consumer with 5% market share in the global beauty sector [1]. Several harmful chemicals including synthetic colourants are very commonly used as ingredients in these products. Synthetic colourants may possibly have an adverse effect on human health causing allergy, nausea, dermatitis, and drying of the skin. They can be carcinogenic and even fatal[2, 3, 4].The synthetic pigments not only have direct impact on human health only , but may also adversely affect the environment .This has made people concerned about the use of natural pigments. Many governments also have imposed restrictions on use of synthetic colourants [5]. Besides this, pigment and dye effluents from various industries like cosmetics and pharmaceuticals augments micro pollutants and contaminate aquatic atmospheres. Most of such contaminants are generally non-biodegradable or on degradation produce carcinogenic composites [6, 7].Therefore the best alternative to protect the environment, as well as human health, is use of natural pigment and dyes.

Recently, bacterial pigments are emerging as a form of natural pigment that has potential to meet existing market demands without inducing adverse effects on human health as well as environment [8]. Furthermore, bacterial pigments are more advantageous as compared to other natural sources such as plants or minerals, as bacteria are easily available and may be cultured in large scale bioreactors, can be engineered easily and also may be optimized to obtain higher yield. Apart from this simple and easy downstream processing, low maintenance and no requirement of large arable lands and labor, unlike plant pigments, makes them attractive source for pigment production. Moreover for

cultivation and processing, food and agricultural wastes may be used as substrates encouraging waste minimization [9].Bacterial pigments are also proved to exhibit different bioactivities like antimicrobial, antioxidant, anti-inflammatory, and anti-carcinogenic etc. which makes them best suitable for food, pharmaceutical as well as cosmetic industries [10].Since bacterial pigments are mostly intracellular, the loss of pigment during extraction and purification process results in a relatively lower net yield than extracellular pigments or other plant pigments. Therefore, selection of best suitable extraction method is critical. Several methods are commonly used for intracellular pigment extraction which comprises use of organic solvents, homogenization, solid-phase extraction, freeze-thaw method, ultrasonication, use of inorganic acids, soxhlet extraction method, and others [11]. Among all, solvent extraction using organic solvents such as ethanol or methanol is modest and cost-effective method. These methods may be employed exclusively or in suitable combinations with other methods keeping in view their availability, simplicity, and production cost for higher yields. In present investigation, an attempt has been made to explore bacterial pigments in cosmetic formulations. The work was accomplished by planning main objectives as to isolate pigmented bacteria, to extract as well as to analyze their applicative potential for use in cosmetic formulations. This study reveals better applicative potential in cosmetics with greater efficacy and added value while diminishing the risk of toxicity and environmental hazard. To the best of our knowledge, this is the first report describing use of bacterial pigments as natural colorants in cosmetic formulations.

MATERIAL AND METHODS

Test chemicals

The bacteriological culture media were procured from Himedia, India. Solvents like ethanol, methanol, chloroform acetone as well as other chemicals were obtained from Loba Chemie, India.

Collection of sample

The water sample was collected from lake, near Satara college of Pharmacy,Degaon, Satara, Maharashtra. (At altitude 17.631613°, longitude 74.049914°).Approximately 100 ml sample was collected from the lake in sterile glass bottle and was immediately processed within 8 hrs of collection.

Isolation of pigment-producing bacteria

Collected water sample was serially diluted up to 10^{-9}. From each dilution 0.1ml of sample was spread on sterile nutrient agar plate. The plates were incubated at 37°C for 48 hrs and were observed for growth after incubation. Only the pigmented bacterial colonies were selected and were used for further studies. The pure cultures of the strain were obtained by subculturing them with quadrant streaking method on sterile nutrient agar plates with 2.5 % (w/v) of agar.

Bacterial cell harvesting

Multiple nutrient agar plates were inoculated with pure cultures of pigment producing bacteria by streak plate technique and were incubated at 37°C for 48 – 72 hrs, when pigment intensity was observed to be visually highest and constant; pigments were subjected for extraction process.

Pigment extraction

Different solvents like ethanol, methanol, acetone and chloroform were screened for extraction of pigments wherein methanol was found to be most suitable. Pigment extraction process was carried out by dissolving whole of the harvested biomass in 50 mL of 100 % methanol (v/v) assisted by vortexing to confirm a homogenous solution. The pigment appeared to be intracellular since centrifuged broth exhibited no color extraction. The suspended cells of isolated bacterial strains were then lysed by ultrasonication treatment in a bathsonicator (Digital UltrasonicCleanerLCMU-2, Labman Scientific Instruments, Chennai, India) for 30 min. After sonication, mixture was centrifuged at 10,000 rpm at room temperature for 20 min to obtain a colorless pellet and colored supernatant[12,13].This was filtered by membrane fitter. Thus obtained methanol solution along with extracted pigment was then air-dried for 3 days in a dust-free environment. The dried pigment residues left after evaporation were suspended in the solvent and then re-evaporated, this step was repeated 2-3 times so that pure pigments were obtained. Finally, partially purified pigments were stored at low temperature until their application.

Formulation of cosmetics:

Powdered rouge, eyeshadow, lipsticks were formulated as per method described wherein extracted bacterial pigments were used as colourants. The ingredients used in the formulation were as per given in table 1, 2 and 3 respectively.

Formulation of rouge

Perfume was mixed properly with maize starch and the mixture was kept covered for half an hour. The remaining ingredients were mixed thoroughly and sieved through fine muslin. Extracted pigments were added to this mixture and formulation was stored in a suitable container.

Table1.Formulation table for Rouge

Sr. no	Ingredients	Quantity given	Quantity taken
1	Zinc stearate	14.5 gm	1.03 gm
2	Maize starch	14.5 gm	1.03 gm
3	Talc powder	55.0 gm	3.92 gm
4	Pigment	14.0 gm	1.0 gm
5	Perfume	2.0 gm	0.14 gm

Formulation of eyeshadow

The Perfume was mixed with the talc powder & was kept covered. The remaining ingredients were mixed thoroughly & mixed with the perfume talc mixture. In the above mixture, the pigment was added as a colorant. The product was stored in suitable container.

Table2.Formulation table for eye shadow

Sr. No	Ingredients	Quantity given	Quantity taken
1	Kaolin	20 gm	0.2 gm
2	Zinc stearate	7.5 gm	0.75 gm
3	Talc powder	32.5 gm	3.2 gm
4	Pigment	15 gm	1.5gm
5	Perfume	q.s	q.s

Formulation of lipstick

Lipstick was formulated as per general method. In brief; all ingredients namely white bees wax, cetyl alcohol, petroleum jelly were melted together in a porcelain dish on water bath. The extracted bacterial pigment was mixed with castor oil and heated. Both phases were mixed together at same temperature. Perfume was added into above mixture. Then mixture was poured into lipstick mould and the mould was kept in refrigerator for 15 min. After solidification the lipstick was removed from the mould and were fitted in a lipstick container and used for further evaluation [14].

Table 3.Formulation table for lipstick

Sr. No	Ingredients	Quantity given	Quantity taken
1	Castor oil	6 ml	6 ml
2	White bees wax	3 gm	3 gm
3	Cetyl alcohol	0.5 gm	0.5 gm
4	Petroleum jelly	0.5 gm	0.5 gm
5	Color pigment	2 ml	2 ml
6	Perfume	q.s	q.s

EVALUATION TESTS

Evaluation of rouge

Color

Formulated rouge was checked for color.

Color dispersion

This test was performed using a microscope.

Pay-off test

Pay-off means adhesion characteristics with powder puff were tested on the skin. [3]

Evaluation of eyeshadow

Color

Color of the formulated eyeshadow was checked.

Melting point test

It is determined by capillary tube for good application and storage point of view.

Texture

Texture of the eyeshadow was checked [15].

Evaluation of lipstick

Melting point:

Melting point determination helps to indicate limit of safe storage. Capillary tube method was made use of for determination of melting point of formulated lipstick.. The capillary was filled, kept in capillary apparatus and observed the product for its melting. The procedure was repeated thrice and the melting point ratio was observed for prepared formulations.

Force of application:
It is performed for comparative measurement of the force to be applied during its application. A piece of coarse brown paper was kept on a shadow graph balance and lipstick was applied at 45º angle to cover a 1 sq. inch area until fully covered. The pressure reading is an indication of force of application.
Colour and Texture:
Formulated lipsticks were checked for colour, glossy appearance and smooth texture.
Surface anomalies:
This was studied by the surface defects, such as no formation of crystals on the surfaces, no contamination by fungi etc.

RESULTS AND DISCUSSION:
Isolation of pigment-producing bacteria
The sample obtained was serially diluted and spread on sterile nutrient agar plates and incubated at 37°C for 72 hours. A mixture of pigment producers and non-pigment producers were obtained on plate (Fig 1).Amongst these two morphologically different pigmented colonies were selected and coded as OP and YP strain, producing orange and yellow pigments respectively (Fig 1).

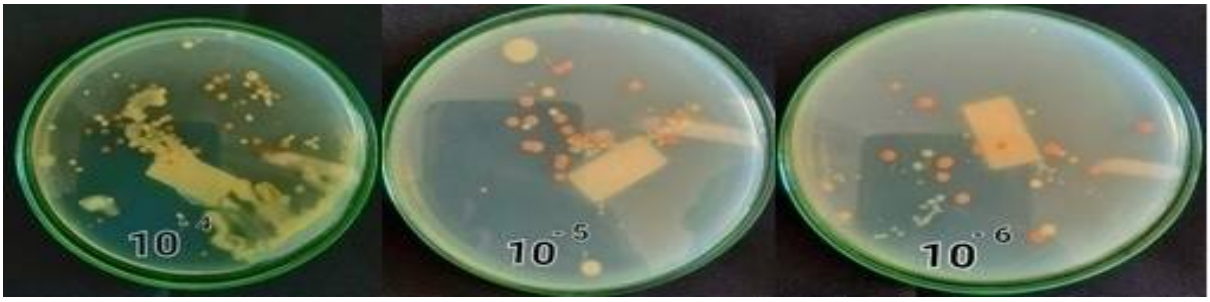

Fig 1: Pigment producers and non-pigment producers obtained on nutrient agar plate

Production of pigments
The production of orange and yellow pigments was carried out by growing the cultures on multiple nutrient agar plates for three days as shown in Fig 2.

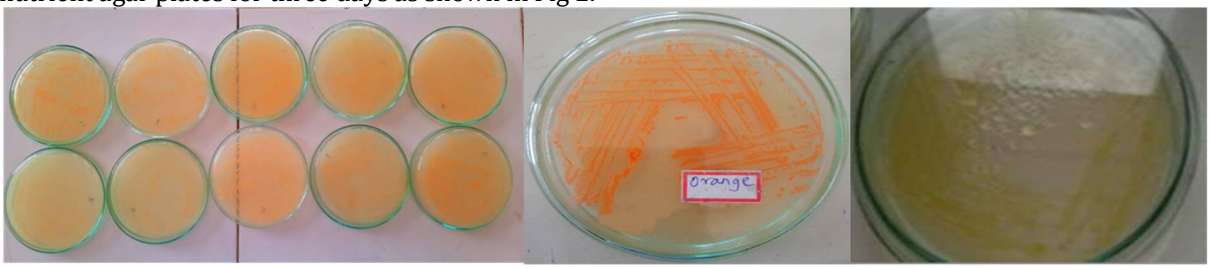

Fig 2: Nutrient agar showing cultivated pigmented bacteria

Extraction of bacterial pigments
The pigment producing bacteria were harvested by centrifugation at 10,000rpm for 20 mins. The supernatants were discarded and pellets were resuspended in methanol for extraction of pigments. The mixture was vortexed and the suspension after sonication was centrifuged at 10,000rpm for 20 mins. Supernatant liquid with orange color was collected and colorless bacterial pellet settled at the bottom of centrifuge tube was discarded. Finally, the supernatant was filtered through a membrane filter, dried and stored at lower temperature.

Fig 3: Extracted orange and yellow pigments from bacterial isolates

Cosmetic formulations using bacterial pigments:
Various cosmetic formulations like rouge, eyeshadow and lipstick were prepared as per methods mentioned above and are as shown in Fig.4

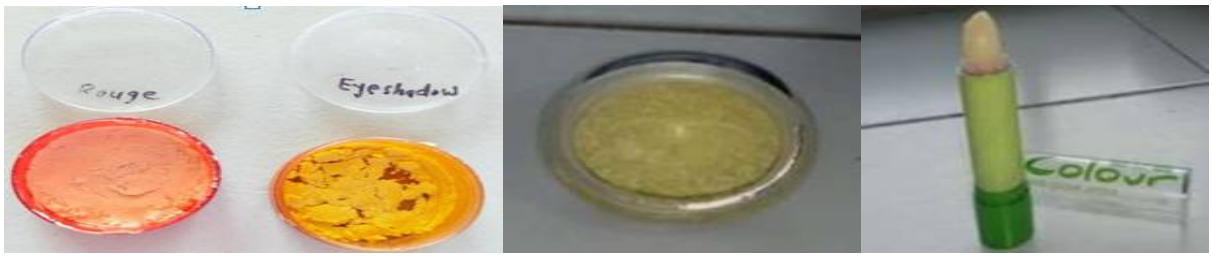

Fig4: Formulated rouge, eye shadow and lipstick

Evaluation of rouge:
Color
Formulated rouge was checked for color. The observed rouge was orange in color.
Color dispersion
This test was performed by using a microscope. Size of color particles was found to be less than 50 um. Colour was found to be properly distributed.
Pay-off test
Pay-off means adhesion characteristic with powder puff should be tested on skin. The results revealed good adhesion.
Evaluation of eyeshadow:
Color
Formulated eyeshadow was checked for color. The observed eyeshadow was orange in color.
Melting point
It was determined by capillary tube method for good application and storage point of view. Melting point of eye shadow was found to be 53°C.
Texture
Texture of the eye shadow was smooth.
Evaluation of lipstick:
Melting point
The melting point of formulated lipstick was observed to be 63^0C.
Force of application
It is the test for comparative measurement of the force to be applied for application of lipstick formulation. The force of application was found to be good.
Color and texture
Formulated lipstick was checked for color, glossy and smooth texture and was found to be satisfactory.
Surface anomalies
It was studied by the surface defects, such as no formation of crystals on the surfaces, no contamination by fungi etc. No defect (s) was observed on the surface.

DISCUSSIONS
The present study was designed to assess the potential application of bacterial pigments in cosmetic formulations. Pigment producing bacteria are ubiquitously distributed and have been reported to be present in diverse ecological niches. Diazotropic red pigmented vibrios were isolated from mangrove rhizosheres [16].Gavrish *et al*. [17], identified three novel species of *Brevibacteria,* all known to produce orange pigment. The yellow pigment producing *Flavobacterium* was also reported [18], which has over hundred different strains with exceptional environmental niche heterogeneity showing distribution in soil as well as aquatic habitats. In a recent study, Meddeb-Mouelhi, F[13]reported a novel antioxidant pigment produced by a photochromogenic *Microbacterium oxydans* FJM1.Carotenoid production by a novel isolate of *Microbacterium paraoxydans* also reported by others [19]. In another study, Goswami and Bhowal [20] reported red pigment production from a novel strain of *Bacillus* species. And *Serratia marcescens* as well as *Erwinia stewartii*. Sinha *et al* reported red and light orange pigment production by *Serratia* (21) whereas Mohammadi *et al.*[22]reported pigment production by *Erwinia*. The chemical nature of the extracted pigment influences choice of organic solvent and yield directly. Carotenoids are lipophilic in nature and are soluble in organic solvents, like chloroform, acetone, methanol, ethanol etc. [23]. Different solvents like chloroform, ethanol, petroleum ether, etc were screened for extraction of pigments from bacterial species, in various studies [24]. Pigments were extracted by different solvents

like acetone, ethyl acetate, chloroform, and methanol at different concentrations. There were no pigment extraction in chloroform and ethyl acetate solvent. Acetone and methanol solvents, can extract the pigment from the cell. But highest extraction of pigment was shown in methanol [25].Our work is in accordance with this report. Three different cosmetics were formulated and evaluated. The results of evaluation tests exhibited that the melting point of the prepared lipstick formulation is 63^{o}C, The force of application for prepared formulations was determined and noted to be good. Different red shades of lipstick formulations like Crimson red, Scarlet red and Raspberry red may also be prepared by using varying concentration of colored pigment. Prepared Rouge, Eye shadow and lipstick were subjected to different evaluation tests. The results of the evaluation tests revealed that rouge formulation exhibited proper distribution of color along with good adhesion property as noted in the payoff test. The eye shadow preparation showed orange color with a melting point of 53^{o}C and exhibited smooth texture. Lipstick also has shown satisfactory results after evaluation tests. Thus, the cosmetic formulations namely rouge, eyeshadow and lipstick, were successfully formulated by using bacterial colored pigments and also evaluated. Many reports are available about use of natural colourants in food, fabrics, pharmaceuticals and in other fields [26, 27] but this is the first report on use of bacterial pigments in cosmetic formulations.

CONCLUSION

In recent era researchers are focusing on natural color producing microorganisms so as to replace the demand for synthetic colors. The search for promising pigment producing microbial strains is a continuous process. The current investigation deals with an approach of developing new sources of natural colors from easily cultivable bacterial species that can be further exploited at larger scale. Orange and yellow pigment producing bacteria were successfully isolated from water sample. The intracellular bacterial pigments were successfully extracted by sonication assisted solvent extraction using methanol as solvent. By using these colored pigments cosmetics were formulated and evaluated. The results of present investigation revealed that the colored pigments obtained from pigmented bacteria can be effectively used as a natural colorant in various cosmetic formulations. To conclude, microbial pigment production is one of the emerging fields of research to demonstrate its potential for various industrial applications. To the best of our knowledge it was the first attempt wherein colored pigments from bacteria were employed as natural colorants in different cosmetic formulations. The use of a natural coloring agent will provide a suitable alternative for synthetic colorants which are known for their side effects.

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Conflicts of interest/competing interests

The authors declare that they have no conflict of interest.

Author's Contribution

Seemadevi Suresh Kadam conceived and designed the experiments .Avinash Ashok Survase conducted experiments and wrote the manuscript. Shivangi Shivraj Kanase involved in reviewing, and editing the manuscript. All authors read and approved the final manuscript.

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Ethics Statement

Not Applicable

Informed Consent

Not Applicable

Data Availability

Not Applicable

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