International Journal of Advance and Applied Research
www.ijaar.co.in
ISSN – 2347-7075 Impact Factor – 8.141
Peer Reviewed Bi-Monthly
Vol.7 No.22 March –April- 2026

Extraction and Evaluation of Bacterial Pigments and Its Application

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DOI - 10.5281/zenodo.19335566

Abstract:

Bacterial pigments are naturally occurring bioactive compounds produced by various microorganisms and are increasingly gaining importance due to their potential cosmetic applications. The widespread use of synthetic pigments has raised concerns related to toxicity and environmental hazards, thereby emphasizing the need for safer and eco-friendly alternatives. The present study was undertaken to extract and evaluate bacterial pigments and to assess their physicochemical and biological properties with respect to cosmetic applications. Pigment-producing bacterial isolates were screened and cultivated under optimized conditions. Pigments were extracted using appropriate solvent extraction methods and evaluated for their physicochemical characteristics, including color intensity, solubility, stability, and spectrophotometric properties. The biological activities of the extracted pigments were assessed through antimicrobial and antioxidant assays. The results demonstrated that the bacterial pigments exhibited significant bioactivity, stability. The findings of this study highlight the importance of bacterial pigments as promising natural compounds and support their potential application in cosmetics as safe alternatives to synthetic pigments.

Keywords: Bacterial Pigments, Natural Bioactive Compounds, Pharmaceutical Applications, Spectrophotometric Analysis, Pigment Stability, Antimicrobial Assay, Antioxidant Activity.

Introduction:

Microorganisms are an important source of structurally diverse and biologically active compounds. Among them, bacterial pigments have gained significant attention due to their wide range of industrial and pharmaceutical applications. Various bacteria such as *Salinicoccus, Pseudomonas*, *Micrococcus* and *Rhodococcus* are known to produce pigments like prodigiosin, pyocyanin, carotenoids, and other coloured metabolites. These pigments are secondary metabolites that help microorganisms survive under environmental stress conditions such as UV radiation, oxidative stress, and extreme temperatures (1).

The extensive use of synthetic dyes in pharmaceutical, cosmetic, textile, and food industries has raised serious concerns due to their toxic, mutagenic, and carcinogenic effects (2). Many synthetic colorants are non-biodegradable and contribute to environmental pollution. As a result, there is growing interest in natural pigments derived from microorganisms because they are biodegradable, eco-friendly, and can be produced through controlled fermentation processes (3).

Bacterial pigments possess important biological properties, including antimicrobial, antioxidant, anticancer, and anti-inflammatory activities (4). Prodigiosin from *Salinicoccus* has shown significant anticancer and immunosuppressive properties (5). Pyocyanin produced by Pseudomonas aeruginosa exhibits antimicrobial activity against various pathogens (6). Carotenoid pigments from Micrococcus luteus

are known for their strong antioxidant activity, which protects cells against oxidative damage (7).

Extraction and evaluation of bacterial pigments involve screening pigment-producing strains, optimizing growth conditions, solvent extraction, and physicochemical characterization such as colour intensity, solubility, stability, and spectrophotometric analysis (8). Further biological evaluation through antimicrobial and antioxidant assays helps in determining their pharmaceutical potential (9).

Therefore, the present study aims to extract, characterize, and evaluate bacterial pigments to assess their physicochemical properties and biological activities, highlighting their potential as safe and effective alternatives to synthetic pigments in pharmaceutical and biomedical applications.

Methods And Material:

1. Sample Collection:

Soil, marine water, contaminated soil, and wastewater samples were collected from different locations using sterile containers to avoid external contamination. Soil samples were collected from a depth of approximately 5–10 cm using sterile spatulas and transferred into sterile zip-lock bags or bottles. Marine and wastewater samples were collected in sterile screw-capped bottles. All samples were properly labelled and transported to the laboratory under refrigerated conditions for further microbiological analysis.(3,4,8)

2. Enrichment of Microbial Culture:

One gram of soil sample (or 1 mL of water sample) was inoculated into 50 mL of sterile nutrient broth in a conical flask. The flasks were incubated at 30–37°C for 24–48 hours in a shaking incubator (120–150 rpm) to promote the growth of microorganisms. Enrichment helps to increase the population of pigment-producing bacteria present in small numbers in environmental samples.(1,3,9)

3. Isolation of Pigmented Colonies:

After incubation, a loopful of enriched culture was streaked onto sterile nutrient agar plates using the streak plate method to obtain isolated colonies. The plates were incubated at 30–37°C for 24–48 hours. Distinct colonies showing visible pigmentation (red, yellow, orange, green, etc.) were observed and selected. These colonies were further streaked on fresh agar plates to obtain pure cultures.(1,3,8)

4. Gram Staining and Biochemical Testing:

Pure isolates were subjected to Gram staining to determine their Gram reaction (Gram-positive or Gram-negative) and cellular morphology (cocci or bacilli). Biochemical tests such as catalase test, oxidase test, indole test, citrate utilization test, and sugar fermentation tests were performed to identify and characterize the bacterial isolates. Results were recorded and compared with standard microbiological identification charts.(3,4,9)

5. Cultivation of Selected Isolates:

The selected pigment-producing isolates were inoculated into fresh sterile nutrient broth and incubated at optimized temperature and pH conditions for 48–72 hours. This step ensured sufficient biomass production and enhanced pigment synthesis. The culture was observed for maximum pigment production before proceeding to extraction. (1,4,8,9)

1.Micrococcus 2.Pseudomonas
3.Salinicoccus 4.Rhodococcus

6. Pigment Extraction:

After incubation, the culture broth was centrifuged at 8000–10,000 rpm for 10–15 minutes to separate the cell mass from the supernatant. Depending on whether the pigment was

intracellular or extracellular, the cell pellet or supernatant was used for extraction. A solvent mixture of methanol, ethyl acetate, and dilute HCl in a ratio of 9:1:0.5 mL was added to the biomass. The mixture was vortexed thoroughly and kept under shaking conditions for proper mixing to allow efficient pigment solubilization. The acidic condition helps in better extraction of certain pigments.(1,5,6,17)

7. Filtration and Purification:

The solvent mixture containing dissolved pigment was filtered using Whatman filter paper to remove cell debris and impurities. The filtrate was collected and, if required, further purified using techniques such as repeated solvent washing or column chromatography to obtain a concentrated pigment extract.(4,8,14)

8. Drying of Pigment Extract:

The purified pigment solution was subjected to evaporation using a water bath or rotary evaporator at controlled temperature to remove the solvent. The remaining residue was dried to obtain a stable, fine pigment powder. The dried pigment was stored in sterile airtight containers at low temperature for further physicochemical and biological evaluation.(2,4,8,15)

Physochemical Testing:

1. pH Testing:

The pH stability of the extracted pigment was evaluated to determine its behavior under acidic, neutral, and alkaline conditions. A known quantity of dried pigment was dissolved in a suitable solvent (such as methanol or distilled water). The pH of the solution was measured using a calibrated digital pH meter. To study pH stability, the pigment solution was adjusted to different pH values (for example pH 3, 5, 7, 9, and 11) using dilute HCl and NaOH. The solutions were incubated at room temperature for 24 hours and observed for any change in color intensity, precipitation, or degradation. Stable pigments show minimal color change across a range of pH values, indicating suitability for pharmaceutical formulations.

2. Chromatography (Thin Layer Chromatography – TLC):

Thin Layer Chromatography was performed to check the purity and separation of pigment components. A small amount of pigment extract was spotted on a silica gel-coated TLC plate using a capillary tube. The plate was placed in a developing chamber containing a suitable solvent system (such as chloroform:methanol or ethyl acetate:methanol). The solvent was allowed to rise up to three-fourths of the plate. After development, the plate was removed, air-dried, and observed under visible light or UV light.

3. Temperature Stability Testing:

Temperature stability testing was conducted to evaluate the effect of heat on pigment stability. The pigment solution was divided into separate test tubes and exposed to different temperatures such as 4°C, 25°C (room temperature), 37°C, 60°C, and 80°C for 15–30 minutes. After incubation, the samples were cooled to room temperature and observed for changes in color intensity, turbidity, or precipitation. A stable pigment maintains its original color without significant degradation at various temperatures. This test is important to determine storage conditions and formulation stability.

4. Spectrum Analysis (UV–Visible Spectrophotometry):

UV–Visible spectrophotometric analysis was carried out to determine the absorption characteristics of the pigment. The pigment was dissolved in a suitable solvent, and the solution was placed in a quartz cuvette. The absorbance was recorded using a UV–Visible spectrophotometer over a wavelength range of 200–800 nm. The wavelength at which maximum absorption (λmax)

occurred was noted. The absorption peak confirms the presence of specific pigment compounds and helps in their characterization. The intensity of absorbance also indicates pigment concentration and purity.

5. Antimicrobial Assay:

The antimicrobial activity of the test sample was evaluated by the disc diffusion method under aseptic conditions. Sterile nutrient agar medium was prepared according to standard procedure and poured into sterile Petri plates. The plates were allowed to solidify at room temperature to obtain a smooth and uniform agar surface. An 18–24 hour old fresh bacterial culture was selected for the test. A sterile cotton swab was dipped into the bacterial broth culture, excess culture was removed by pressing against the tube wall, and the swab was evenly spread over the entire surface of the agar plate in three directions to obtain a uniform bacterial lawn.

Sterile filter paper discs were prepared and soaked in the test sample solution for about 5–10 minutes to allow proper absorption. Using sterile forceps, the soaked discs were carefully placed on the surface of the inoculated agar plates and gently pressed to ensure complete contact with the medium. The discs were placed at equal distances from each other to avoid overlapping of zones. A control disc (without sample or with standard antibiotic) may also be placed for comparison.

The plates were then incubated in an inverted position at 37°C for 18–24 hours. After incubation, the plates were examined for the appearance of a clear circular area around the discs. This clear region, known as the zone of inhibition, indicates the inhibition of bacterial growth by the test sample. The diameter of the zone of inhibition was measured in millimeters using a ruler or Vernier caliper. The presence and size of the zone were used to determine the antimicrobial effectiveness of the test sample. A larger zone of inhibition indicated stronger antimicrobial activity, whereas a smaller or absent zone suggested weak or no activity against the tested microorganism.

6. Blush preparation:

Blush was prepared using bacterial pigments as a natural colorant for cosmetic formulation. The cosmetic base was prepared by mixing talc powder and corn starch in a clean mortar and pestle to obtain a smooth and uniform powder. A small amount of dried bacterial pigment obtained from pigment-producing bacteria such as Rhodococcus species and Micrococcus species was added gradually to the base and mixed thoroughly to achieve the desired shade of color. A few drops of glycerin or rose water were added as a binding agent to improve the texture and spreadability of the formulation. The mixture was blended properly until a fine and homogeneous blush powder was formed. Finally, the prepared blush was transferred into a cosmetic container, pressed gently, and allowed to dry at room temperature before evaluation for colour, texture, and skin compatibility.(18,19,20)

Fig: Blush Preparation

7. Patch Test for Skin Irritation:

The skin irritation test of the formulated lip balm was performed at the college laboratory level to evaluate its safety for topical application. A small quantity of the prepared lip balm was applied to a small area (approximately 1 cm^2) on the inner forearm of selected healthy student volunteers after obtaining their consent. Prior to application, the test area was cleaned with distilled water and allowed to dry properly.

The lip balm was gently applied on the marked area and left uncovered under normal room temperature conditions. The applied site was

observed at regular intervals for a period of 24 hours. The skin was carefully examined for any visible signs of irritation such as redness, itching, swelling, rashes, burning sensation, or any other abnormal reaction. All observations were recorded systematically for further evaluation.

Results And Discussion:

Pigmented colonies were isolated and screened from environmental samples, yielding four pure pigment-producing cultures with distinct characteristics and high extraction efficiency.

1. Isolation and Identification of Pigment-Producing Bacteria: The selected isolates exhibited vivid pigmentation: *Pseudomonas* sp. (blue-green diffusible), *Micrococcus luteus* (bright yellow intracellular), *Rhodococcus* sp. (orange-red intracellular), and *Salinicoccus sp.* (pink-orange extracellular). Gram staining confirmed morphology, while comprehensive biochemical tests, including sugar fermentation, MR-VP, and tryptone broth tests, provided genus-level identification (Table 1).

MORPHOLOGICAL CHARACTERISTICS

Parameter	*Pseudomonas*	*Micrococcus*	*Rhodococcus*	*Salinicoccus*
Gram reaction	Gram negative (–)	Gram positive (+)	Gram positive (+)	Gram positive (+)
Shape	Rod	Cocci	Cocci	Cocci
Arrangement	Single	Tetrads	Rod / Coccoid	Tetrads / clusters
Motility	Motile	Non-motile	Non-motile	Non-motile
Spore formation	Absent	Absent	Absent	Absent
Pigment production	Blue-green	Yellow	Cream to orange	Pink to red
Colony shape	Circular	Circular	Circular	Circular
Colony margin	Entire	Entire	Entire	Entire
Colony elevation	Flat / Slightly raised	Convex	Convex	Convex
Colony opacity	Opaque	Opaque	Opaque	Opaque
Colony consistency	Smooth	Smooth	Smooth	Smooth

EXPANDED BIOCHEMICAL CHARACTERISTICS

Test	*Pseudomonas*	*Micrococcus*	*Rhodococcus*	*Salinicoccus*
Catalase	Positive	Positive	Positive	Positive
Oxidase	Positive	Positive	Variable / Negative	Positive
Indole (*Tryptone broth*)	Negative	Negative	Negative	Negative
Methyl Red (MR)	Negative	Negative	Negative	Negative
Voge–Proskauer (VP)	Negative	Negative	Negative	Negative
Citrate utilization	Positive	Positive	Positive	Positive
Urease	Negative	Variable	Positive	Positive
Nitrate reduction	Positive	Variable	Positive	Negative
TSI reaction	K/K (*Non-fermenter*)	K/K	K/K or weak A/K	A/A

SUGAR FERMENTATION PROFILE

Sugar	*Pseudomonas*	*Micrococcus*	*Rhodococcus*	*Salinicoccus*
Glucose	Negative (oxidative only)	Negative	Weak Positive	Positive
Lactose	Negative	Negative	Negative	Negative
Sucrose	Negative	Negative	Variable Positive	Positive
Mannitol	Negative	Negative	Positive	Positive
Maltose	Negative	Negative	Positive	Positive

Table 1: Morphological and Expanded Biochemical Characteristics

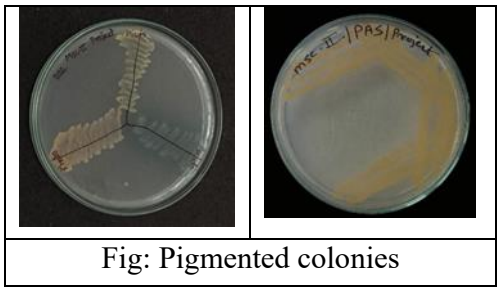

Fig: Pigmented colonies

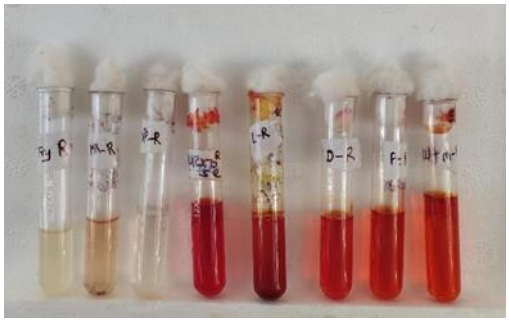

Fig : Biochemicals (*Rhodococcus sp.)*

Fig : Biochemicals (*Micrococcus sp.)*

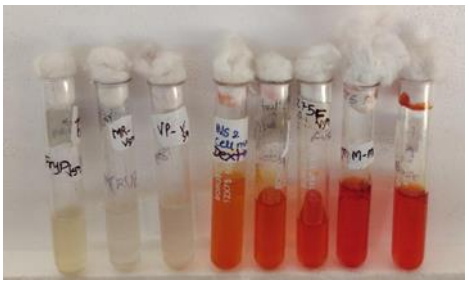

Fig: Biochemicals (*Salinicoccus*)

2. Pigment Production and Extraction Yields:

Optimized cultivation (Table 2) produced peak pigmentation at stationary phase (OD600 1.95–2.82). Solvent extraction from 100 mL cultures yielded stable powders: *Rhodococcus sp.* (1.85 ± 0.12 mg/mL, highest), *Micrococcus sp*. (1.42 ± 0.09 mg/mL), *Salinicoccus* sp. (1.28 ± 0.11 mg/mL), *Pseudomonas* sp. (0.92 ± 0.08 mg/mL). Gram-positive isolates showed 40–100% higher

yields than Gram-negative due to intracellular accumulation

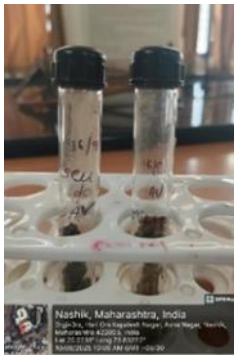

Fig: bacterial Pigment powder

Bacterial Isolate	Pigment Type	Pigment Yield
Rhodococcus sp.	Orange carotenoid	1.85 ± 0.12 mg/mL
Micrococcus sp.	Yellow carotenoid	1.42 ± 0.09 mg/mL
Salinicoccus sp.	Pink carotenoid	1.28 ± 0.11 mg/mL
Pseudomonas sp.	Pyocyanin	0.92 ± 0.08 mg/mL

Fig: Pigment yield

3. Physicochemical Characterization:

TLC Analysis: Thin Layer Chromatography was performed using chloroform: methanol (9:1) as the solvent system.

Pigment Source	Rf Value	Observation
Micrococcus sp.	0.68	Single yellow spot
Pseudomonas sp.	0.65	Blue-green spot
Rhodococcus sp.	0.52	Orange spot
Salinicoccus sp.	0.45	Pink spot

Fig : TLC

pH Stability: Micrococcus and Rhodococcus retained 92–98% colour across pH 3–11; Salinicoccus stable pH 5–10; Pseudomonas optimal pH 6–8.

Bacterial Pigment	Stability Range	Observation
Micrococcus sp.	pH 3–11	Colour remained stable
Rhodococcus sp.	pH 3–11	Minimal colour change
Salinicoccus sp.	pH 5–10	Slight fading at extreme pH
Pseudomonas sp.	pH 6–8	Maximum stability

Fig : stability testing

These results indicate that most pigments are stable across a broad pH range, making them suitable for pharmaceutical formulations.

Temperature Stability:

All pigments >95% stable at 4–60°C; Rhodococcus/Micrococcus retained 88% at 80°C.

Temperature	Observation	Result
−20°C	Pigment colour remained unchanged with no precipitation	Highly stable under freezing conditions
3°C	No visible colour change observed	Stable during refrigerated storage
24°C	Pigment retained original colour intensity	Stable at room temperature
37°C	Slight reduction in colour intensity in some pigments, but no major degradation	Moderately stable at elevated temperature

Fig : Temperature Analysis

UV-Vis Spectra: Spectral analysis was performed in the wavelength range 200–800 nm

λmax: Pseudomonas 691 nm, Micrococcus 465 nm, Rhodococcus 480 nm, Salinicoccus 440/530 nm.

Pigment Source	Λmax (nm)	Pigment Type
Pseudomonas sp.	691 nm	Pyocyanin
Micrococcus sp.	465 nm	Carotenoid
Rhodococcus sp.	480 nm	Carotenoid
Salinicoccus sp.	440 / 530 nm	Carotenoid

Antimicrobial Assay:

After incubation, a distinct clear circular zone was observed around the disc containing the test sample, while the surrounding area showed visible bacterial growth. This clear region, known as the zone of inhibition, indicates that the test sample was able to inhibit the growth of the microorganism. The presence of a measurable zone confirms that the sample possesses antimicrobial activity against the tested organism. Furthermore, a larger diameter of the zone of inhibition suggests stronger antimicrobial effectiveness, whereas a smaller zone indicates moderate activity. If no clear zone had been observed, it would indicate that the test sample has no antimicrobial effect against the selected microorganism under the given experimental conditions.

Patch Test of Blush:

Skin Irritation Test:

The formulated lip balm was evaluated for skin irritation at the college laboratory level using healthy student volunteers. After application on the inner forearm and observation for 24 hours, no visible signs of redness, itching, swelling, rashes, burning sensation, or inflammation were observed in any of the participants. The test area remained normal in appearance without discomfort or allergic reaction.

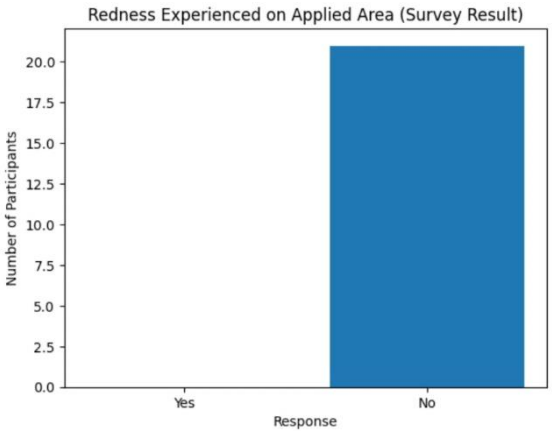

Fig: Google survey of test

Conclusion:

The present study focused on the extraction, characterization, and biological evaluation of bacterial pigments to assess their cosmetic potential. Pigment-producing bacteria such as *Salinicoccus sp. Pseudomonas sp. and Micrococcus sp.* were found to produce bioactive pigments with significant physicochemical stability and . The extraction process using suitable organic solvents resulted in pigments with good colour intensity, solubility, and stability under varying pH and temperature conditions. Spectrophotometric analysis confirmed characteristic absorption peaks, indicating the presence and purity of specific pigment compounds. The stability of these pigments is particularly important for pharmaceutical applications, as it ensures consistent performance, longer shelf life, and effectiveness in formulations. Furthermore, antimicrobial and antioxidant assays demonstrated that the extracted pigments possess notable biological activities, including inhibition of microbial growth and free radical scavenging properties, which may contribute to infection control and protection against oxidative stress.

Overall, the findings highlight that bacterial pigments are not only natural colouring

agents but also promising bioactive compounds with potential applications. In comparison to synthetic dyes, which are often associated with toxicity, carcinogenicity, and environmental hazards, microbial pigments offer advantages such as biodegradability, eco-friendly production through fermentation, and enhanced biocompatibility. These characteristics make them suitable candidates for use in pharmaceutical and biomedical formulations. However, before large-scale industrial application, further research is required to perform detailed toxicity studies, structural characterization, purification, and in vivo evaluations to ensure safety and efficacy. In conclusion, bacterial pigments represent a sustainable and innovative alternative to synthetic pigments and hold significant promise for future development in the pharmaceutical field.

References:

1. Venil CK, Zakaria ZA, Ahmad WA. Bacterial pigments and their applications. Process Biochemistry. 2013;48(7):1065–1079.
2. Downham A, Collins P. Colouring our foods in the last and next millennium. International Journal of Food Science & Technology. 2000;35:5–22.
3. Malik K, Tokkas J, Goyal S. Microbial pigments: a review. International Journal of Microbial Resource Technology. 2012;1(4):361–365.
4. Rao MP, Xiao M, Li WJ. Microbial pigments: sources and applications in pharmaceuticals. Applied Microbiology and Biotechnology. 2017;101:2153–2163.
5. Darshan N, Manonmani HK. Prodigiosin and its potential applications. Journal of Food Science and Technology. 2015;52(9):5393–5407.
6. Lau GW, Hassett DJ, Ran H, Kong F. The role of pyocyanin in Pseudomonas aeruginosa infection. Trends in Molecular Medicine. 2004;10(12):599–606.
7. Krinsky NI. The antioxidant and biological properties of carotenoids. Annals of the New York Academy of Sciences. 1998;854:443–447.
8. Tuli HS, Chaudhary P, Beniwal V, Sharma AK. Microbial pigments as natural color sources: current trends and future perspectives. Journal of Food Science and Technology. 2015;52:4669–4678.
9. Ahmad WA, Ahmad WY, Zakaria ZA, Yusof NZ. Applications of bacterial pigments in biotechnology. International Journal of Molecular Sciences. 2012;13:169–185.
10. National Publications of Biological Sciences. (n.d.). Microbial pigments: Production and their applications in various industries. Retrieved from https://www.npcbs.com/articles/microbial-pigments-production-and-their-applications-invarious-industries.
11. Kumar, A., Vishwakarma, H. S., Singh, J., Dwivedi, S., & Kumar, M. (n.d.). Microbial pigments: Production and their applications in various industries. Department of Biochemical Engineering and Food Technology, Harcourt Butler Technological Institute, Kanpur, Uttar Pradesh, India.
12. Kumar, A., Vishwakarma, H. S., Singh, J., Dwivedi, S., & Kumar, M. (n.d.). Microbial pigments: Production and their applications in various industries. Retrieved https://www.jpchs.com/articles/microbial-pigments-production-and-their-applications-invarious-mdustries.pdf from
13. Department of Applied Sciences, Faculty of Humanities and Sciences, Sri Lanka Institute of Information Technology, & Sri Lanka Institute of Nanotechnology. (n.d.). Microbial pigments: Production and

applications. Retrieved from https://www.frontiersin.org/journals/sustainable-food-systems/articles/10.3389/fsufs.2021.603892/pdf

14. Frontiers in Sustainable Food Systems. (2021). Microbial pigments: Production and their applications in various industries. Retrieved from https://www.frontiersin.org/journals/sustainable-food-systems/articles/10.3389/fsufs.2021.603892/pdf
15. Microbial pigments: Major groups and industrial applications. (n.d.). Retrieved from https://pdfs.semanticscholar.org/f7ae/202e1bbe3b0c554e3ed6b44e963ea7269019
16. Innovation Info. (n.d.). Industrial application of bacterial pigments. Journal of Applied Microbiology and Biochemistry Research. https://www.innovationinfo.org/articles/JAMBR/JAMBR-167.pdf Retrieved from.
17. Production of antimicrobial blue green pigment Pyocyanin by marine Pseudomonas aeruginosa by Osama M. Darwesh, Khouloud M. Barakat, Magdy Z. Mattar, Shawky Z. Sabae & Sahar H. Hassan https://www.researchgate.net/publication/338127721_Production_of_antimicrobial_blue-green pigment Pyocvanin by marine Pseudomonas aeruginosa (2019).
18. World Health Organization. Guidelines on Microbial Pigments and Natural Colorants in Cosmetics.
19. U.S. Food and Drug Administration. Cosmetic Safety and Color Additives Guidance.
20. Harry's Cosmeticology. Wilkinson, J.B. & Moore, R.J., Cosmetic Science and Technology.