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Exploration of pigment producing bacteria from different fruits and vegetables

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Abstract
Different fruits and vegetables were used to isolate pigmented microorganisms. *Viz.* papaya, tomato, spinach, beetroot, strawberries and black grapes capable of producing pigments. Nine coloured bacterial isolates were discovered in various fruits and vegetables, and they were used to analyse and extract pigment. The Bergey's Manual of Determinative Bacteriology described them and the bacterial isolates were identified on the basis of biochemical test kit KB003 (Hi25^{TM} Media$^{®}$) which is a comprehensive test framework. The extracts were prepared using four different solvents *viz* chloroform, ethyl alcohol, methanol and acetone where chloroform and methanol was screened as a best among all because of its maximum capability to produce pigmented extracts and the maximum absorption of each sample was determined by UV spectrometer. Antimicrobial studies, MIC and MBC were performed using standard methods.

Keywords: Bio-colour, pigmented bacteria, antimicrobial, food-pigments

1. Introduction
Some bacteria have microbial colours as a distinguishing trait that may aid in identification. Bacterial pigments have intriguing opportunities for a variety of applications because of their superior biodegradability and increased environmental compatibility. Utilising fermentation technology, numerous microorganisms, such as bacteria, fungi, yeast, and mould, are used to produce different colours in industrial quantities. These microbiological pigments are used widely, mostly in the food, pharmaceutical, and textile sectors. Microbial pigments are employed in a wide range of applications, from food to cosmetics, and exhibit superior biodegradability and environmental compatibility than synthetic pigments. Identification of novel microbiological sources and enhancement of process parameters are two areas of focus for cheap pigment synthesis (Fatima and Anuradha 2022) [5]. Colourants are utilised in a variety of products, such as textiles, food, and cosmetics. These dyes are artificial and extremely hazardous to people. Due of the poisonous and non-biodegradable substances it contains, it is extremely damaging to the environment. Natural colours that are biodegradable and less damaging to humans are increasingly in demand nowadays. The relevance of natural pigments is growing and is of interest to people all around the world. Due to its high adaptability and productivity compared to other sources, this form of pigment is very productive. Pigments can be produced in large quantities on inexpensive substrates, and they occasionally even grow on waste materials. As a result, it also recycles environmental waste (Anzum *et al*. 2022) [1]. In addition to being natural, bacterial pigments have a number of other qualities that make them potentially useful in the textile, food, cosmetic, and pharmaceutical industries. These qualities include antibacterial, antimicrobial, antioxidant, antileishmanial, anticancer, and antitubercular activity. Different colours and hues of bacterial pigments need to be developed in order to meet market demand and replace the dangerous synthetic pigment industry (Podder *et al*. 2020) [16].
Microorganisms produce a variety of pigments, including carotenoids, prodigiosin, melanins, quinones, flavins, monascins, and violacein, among others. Some of these can dissolve in water (Sinha *et al*. 2017) [20]. The commercial value of synthetically produced pigments is declining as a result of many societal and scientific concerns (Koes *et al*. 1994) [10]. Some of the roles played by microbial pigments include UV protection, antioxidant activity, heat and cold resistance, antibacterial and anticancer activity, and nutrient uptake such as iron, nitrogen, and carbon. 2016's Pankaj *et al*. [14]. The benefits of bacterial pigment synthesis include quick

and simple growth in inexpensive culture media, immunity to environmental factors, and the ability to produce colours in a variety of colours. Thus, one of the newer topics of study demonstrating the potential of microbial pigment generation for diverse commercial applications.

The main goal of the research was to identify the best pigment-producing microorganisms from a variety of fruits and vegetables and to assess how well they inhibit human pathogenic bacteria. Bacterial pigment production has several benefits, including quick and simple growth in inexpensive culture media, immunity to environmental factors, and the ability to produce shades of diverse hues. Therefore, one of the newer topics of study to show promise for diverse industrial uses is microbial pigment synthesis.

2. Materials and methods

2.1 Sampling

Samples of various fruits and vegetables were taken from Jammu's local marketplaces in India. Black grapes, Papaya, Strawberries, Spinach, Tomato, and Beetroot were among the several samples gathered. The pigment-producing bacteria that were isolated from these samples were used in the current study.

2.2 Chemicals and media

For the studies, distilled water was used, and aseptic conditions were upheld throughout the entire investigation. It was done using bacterial media like nutrient broth, nutrient agar, salt mannitol agar, tryptone soy agar, and macConkey agar. Nutrient agar and nutrient broth containing glycerol for enhancement of coloured bacteria were employed as the media for enrichment and isolation of the coloured bacteria and were purchased from Hi Media, India. For biochemical assays, a Gramme staining kit and reagents were employed. As solvents for the extraction procedure, methanol, acetone, chloroform, and ethyl acetate were employed.

2.3 Isolation of bacteria

Each sample was thoroughly washed four or five times with tap water, once with distilled water, and once more with a mortar and pestle in distilled water. All the samples were blended in a ratio of 1 g of sample to 10 ml of distilled water. The samples were streaked on sterile nutrient agar plates that also contained glycerol in order to enhance coloured microorganisms. After that, they were incubated for 48 hours at 37°C. The plates were examined for growth after being incubated. Only the pigmented bacterial colonies were selected for additional study. The isolated pigment-producing bacteria were then streaked on sterile NA plates to create pure cultures. (Barth *et al*. 2009) [2].

2.4 Purification and maintainence of pigment producing bacterial isolates

To create a pure culture, all the bacterial colonies that produced colour and had distinct morphologies on the plates were selected and streaked on sterile nutrient agar plates. The colonies were kept as stock cultures for additional research after being sub-cultured on the sterile nutrient agar slants. Cultures were kept in the refrigerator at 4°C. (Qayyum *et al*. 2019)[18].

2.5 Characterization of isolates

All the isolates were characterized on the basis of morphology and biochemical test. The biochemical test kit KB003 (Hi25TM Media®), a complete test framework, was used to identify the bacterial isolates. Each kit includes a total of 20 tests. The tests' foundational idea was the efficient use of the substrate. The organisms undergo metabolic changes during incubation, which are visible as an abrupt colour shift in the media that can be detected visually or after the addition of the reagent. Gramme staining, appearance, growth characteristics on Nutrient agar media, and different biochemical assays suggested in the Bergey's manual were used to identify isolates acquired in pure cultures. (Sinha *et al*. 2017) [20].

2.6 Production of pigment producing bacteria

Colonies of identified cultures were isolated and suspended in nutritional broth containing 2% glycerol in flasks, which were then cultured for 48 hours on a rotary shaker. After 48 hours, the bacteria in the flasks had grown significantly.

2.7 Enrichment of pigment producing bacteria

To enrich the media 2% of glycerol was added to nutrient broth and the flask was inoculated with isolates and incubated at 37ºC at 100 rpm for 3–7 days, until it was heavily pigmented and nearly opaque. (Waghela and Khan. 2018)[22].

2.8 Extraction of pigmented bacterial isolates

The pigment was extracted by using various organic solvents *viz*., ethyl acetate, methanol, chloroform, and acetone. Following incubation, the culture broth was centrifuged at 3000 rpm for 10 minutes. The colourless supernatant was discarded. The pellet was repeatedly vortex-mixed with the various solvents until the particle became colourless. (Priya *et al*. 2017) [17]. to allow the solvent to evaporate, the supernatant was separated, passed through Whatman No. 1 filter paper, and then refrigerated. To produce pure pigments, the dry pigment remnants that remained after evaporation were suspended in the solvent and then re-evaporated 2-3 times.

2.9 Detection of Lambda Max

Since most pigments absorb light at a specific wavelength, spectrophotometric analysis of pigment expression may be monitored. An UV-visible spectrophotometer was used to evaluate the extract between the wavelengths of 400 and 700 nm in order to determine the greatest absorption spectra. (Priya *et al*. 2017) [17].

2.10 Antibacterial activity

By using the well diffusion method and Mueller Hinton agar against four human pathogens, including *Salmonella typhi* (MTCC98), *Pseudomonas aeruginosa* (MTCC74), *Klebsiella pneumonia* (KS19), and *E. coli* from Jammu University, the antimicrobial activity of the extracted pigments was evaluated. In this approach, test microorganisms were placed in petri plates with 20 ml of sterile molten media and allowed to solidify. Next, MHA plates with 100 µl l of test organisms were dispersed on media using an L-shaped spreader. Four wells were drilled into the plates using a 6 mm cork borer after the plates had dried, and 100µl l of extracted pigment was added to each well. For 24 hours, plates were incubated at 37 °C. Zone of inhibition was visible after incubation (Dahiya and Purkayastha, 2012) [4].

2.11 Determination of Minimum inhibitory concentration (MIC)

According to the instructions provided by Kowalska &

Dudek, 2021, the broth dilution method was used to estimate the minimum inhibitory concentration (MIC) of pigment extracts. [11] With a few minor modifications. The extracts were serially diluted twice using nutritional broth. Five test tubes containing a 500 mg/ml extract solution were serially diluted to concentrations of 100, 80, 60, 40, and 20 mg/ml. Each tube received 1 ml of a microbial suspension, which was added, and was incubated at 37 °C for 24 hours. The test extracts were dissolved in DMSO in the control tubes, which contained only the test microorganisms. After incubation, the visual turbidity was noticed and contrasted with the unfavourable control. But a spectrophotometer was used to quantify the suspension's turbidity in the inoculum preparation, and it was then corrected in accordance with 0.5 McFarland standards. ($1x10^8$ CFU/ml). The pigment extracts were classified as having a MIC at the lowest concentration at which turbidity was not seen.

2.12 Determination of Minimum bactericidal concentration (MBC)

The broth dilution approach somewhat modified the Parvekar *et al*. 2020 [15] method for calculating the minimum bactericidal concentration (MBC) of plant extracts. After being incubated at 37 °C for 24 hours, it was shown that the minimum bactericidal concentration (MBC), or lowest extract concentration, killed 99.9% of the bacterial inocula.. 100 l aliquots from each tube that exhibited no discernible bacterial growth after the MBC assessment of the plant extract and an equivalent volume from the control tubes devoid of the extracts were inoculated on Mueller-Hinton agar and incubated at 37 °C for 24 hours. The MBC endpoint is reached when 99.9% of the bacterial population has been eliminated at the lowest antimicrobial agent concentration. This was accomplished by examining agar plates that had been pre- and post-incubated to determine whether or not bacteria were present.

3. Results

3.1 Isolation of pigment-producing bacteria

Six different samples, *viz*., black grapes, papaya, strawberries, spinach, tomato, and beetroot, were gathered from Jammu's local markets and utilised to isolate bacteria that produce colour. Nine bacteria that produce pigments were found and described; they were coloured light yellow, dark yellow, light orange, dark orange, reddish pink, and greenish. The materials were smeared onto nutrient agar plates and left to incubate for 48 hours at 37 °C. On nutrient agar plates, a mixture of pigment producers and non-pigment producers were obtained (Fig.1).

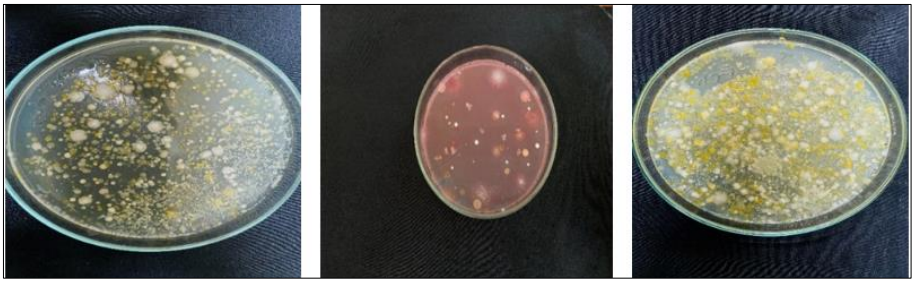


Fig 1: Pigment producers and non-pigment bacterial producers obtained on Nutrient agar plate

3.2 Purification and maintainence of pigment producing bacterial isolates

After 48 h of incubation, all the pigment-producing, morphologically different bacterial colonies that appeared on the plates were picked, and further streaked on sterile nutrient agar plates to obtain a pure cultures incubated at 37°C for 48 hrs. (Fig. 2).

Fig 2: Bacterial isolates (SK1-SK9)

3.3 Characterization of pigment producing bacterial isolates

All the bacterial isolates were characterized on the basis of morphology, Gram's staining, and biochemical assays (Fig 3).

3.3.1 Morphological identification

Colony characteristics were visually assessed *viz*, colony shape, colour, margin, opacity, consistency, elevation and Gram's staining (Table 1).

Table 1: Colony characteristics of selected pigment producing isolates

	Size	Shape	Colour	Opacity	Consistency	Elevation	Gram nature
SK1	2mm	Circular	Dark yellow	Opaque	Mucoid	Flat	Gram +ve
SK2	1mm	Raised	Orange	Opaque	Matte, brittle	Concave	Gram +ve
SK3	1mm	Irregular	Pink	Opaque	Butyrous	Concave	Gram -ve
SK4	2mm	Irregular	Green	Opaque	Slimy,moist	Flat	Gram -ve
SK5	3mm	Circular	Yellow	Translucent	Sticky	Concave	Gram -ve
SK6	1mm	Circular	Light Yellow	Translucent	Butyrous	Concave	Gram +ve
SK7	2mm	Circular	Yellow	Opaque	Sticky	Concave	Gram +ve
SK8	2mm	Circular	Orange	Translucent	Butyrous	Concave	Gram +ve
SK9	3mm	Circular	Yellow	Opaque	Mucoid	Concave	Gram +ve

Gram staining was done using Gram staining kit, and positive and negative bacteria were differentiated as shown in the pics

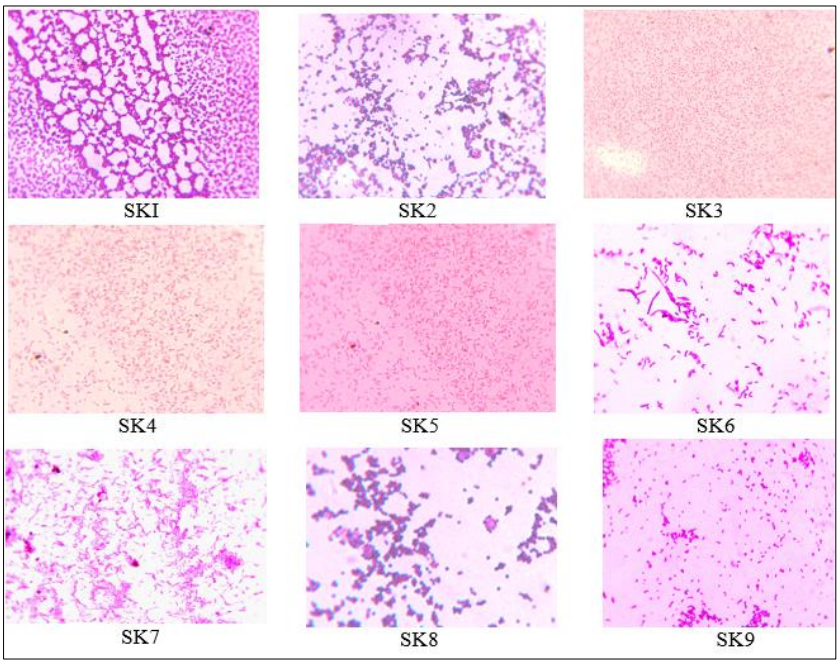


Fig 3: Morphological identification

3.3.2 Biochemical identification: The bacterial isolates were identified on the basis of biochemical test kit KB003 (Hi25™ Media®) which is a comprehensive test framework. Each kit contains a combination of 20 tests (Fig 4) and table 2 showed the positive as well as negative results of respective biochemical tests.

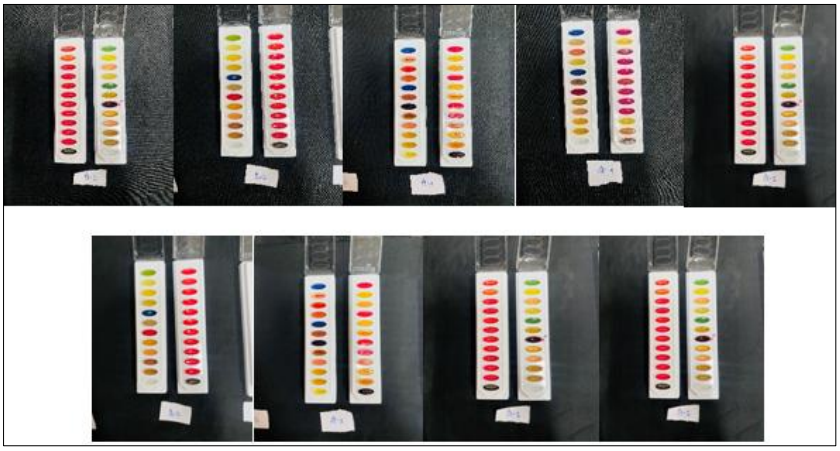


Fig 4: Biochemical identification (SK1-SK9)

Table 2: Biochemical reactions of pigment-producing isolates

S. No	Biochemical Test	Isolates								
		SK1	SK2	SK3	SK4	SK5	SK6	SK7	SK8	SK9
1	ONPG	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve
2	Lysine utilization	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
3	Ornithine utilization	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
4	Urease	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
5	Phenylalamine Deamination	-ve	-ve	_ve	+ve	-ve	-ve	_ve	+ve	-ve
6	Nitrate reduction	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
7	H^2S production	- ve	-ve	-ve	-ve	- ve	-ve	-ve	-ve	- ve
8	Citrate utilization	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve
9	Voges Proskauer's	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve
10	Methyl red	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
11	Indole	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve
12	Malonate utilization	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve
13	Esculin hydrolysis	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve
14	Arabinose	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve
15	Xylose	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve
16	Adonitol	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve
17	Rhamnose	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
18	Cellobiose	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
19	Melibiose	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve
20	Saccharose	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve
21	Raffinose	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
22	Trehalose	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve
23	Glucose	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
24	Lactose	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

3.4 Production of pigment: The development of pigments was carried out by growing the cultures in nutrient broth enriched with 2% glycerol and incubated for 7 days at 37 °C. All the nine isolates showed pigmented growth in nutrient broths (Fig. 5). SK1, SK5, SK6. SK7 and SK9 showed yellow pigment, SK2 showed reddish-orange pigment, SK3 showed pinkish-red pigment, SK4 and SK8 showed green pigment in the nutrient broth resp.

Fig 5. Pigment production in nutrient broth (SK1-SK9)

3.5 Extraction of microbial pigments
The pigment was extracted by using four different solvents. Out of which, the maximum yield was obtained from chloroform and methanol (Table 3.6 & Fig. 6) as compare to others.

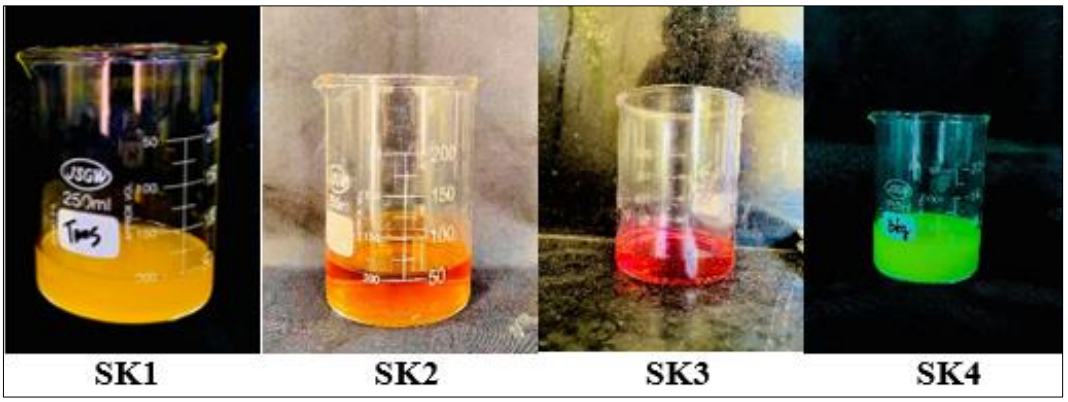


Fig 6: Green, red, yellow and orange pigments extracts

3.6 Yield of crude pigment extract from different bacterial isolates

The mean yield of crude pigment extract obtained from culture broth of different bacterial isolates in different solvents is shown in Table 3.

Table 3: Yield of the crude methanol, ethyl acetate, acetone and chloroform extract of bacterial isolates

Isolates	Yield of Methanol extract(µg/mL)	Yield of Ethyl acetate extract(µg/mL)	Yield of Acetone extract(µg/mL)	Yield of Chloroform extract(µg/mL)
SK-1	1.11	1.41	1.08	1.80
SK-2	1.51	1.09	1.11	1.01
SK-3	1.33	1.61	1.05	3.02
SK-4	1.42	1.25	1.09	2.01
SK-5	1.31	1.03	1.21	2.11
SK-6	1.11	1.14	1.15	1.19
SK-7	1.39	1.42	1.06	1.56
SK-8	1.19	1.06	1.09	1.22
SK-9	1.33	1.10	1.11	2.01

3.7 Ultraviolet -Visible spectroscopy

The maximum absorption of the pigments SK1, SK2, SK5, SK6, SK7, and SK9 was observed between the wavelengths of 440 nm and 500 nm (Fig. 7), which indicates the presence of carotenoids, whereas the maximum absorption of the green pigments SK4 and SK8 was observed in a range between 550 nm and 680 nm (Fig. 8), which indicates the presence of phycocyanin. The greatest absorption peak of Prodigiosin has been shown to be equal to the maximum absorption peak of the pigments SK3 at 520 nm and 540 nm (Fig. 9). Four of the nine pigment extracts that displayed various peaks and hues were chosen for additional research.

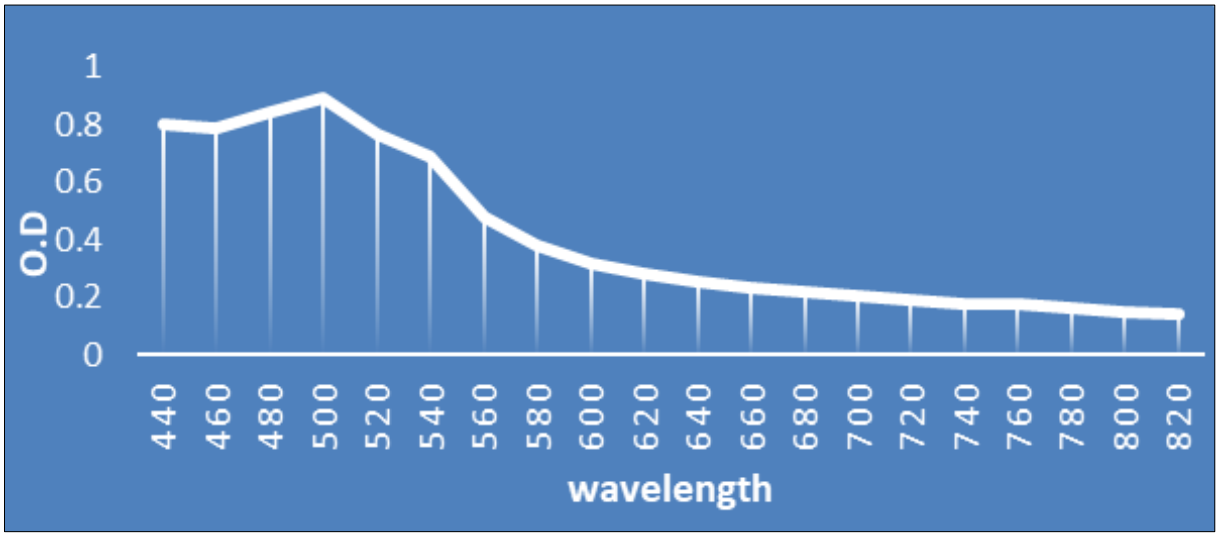


Fig 7: Spectrometric analysis of extracted pigment

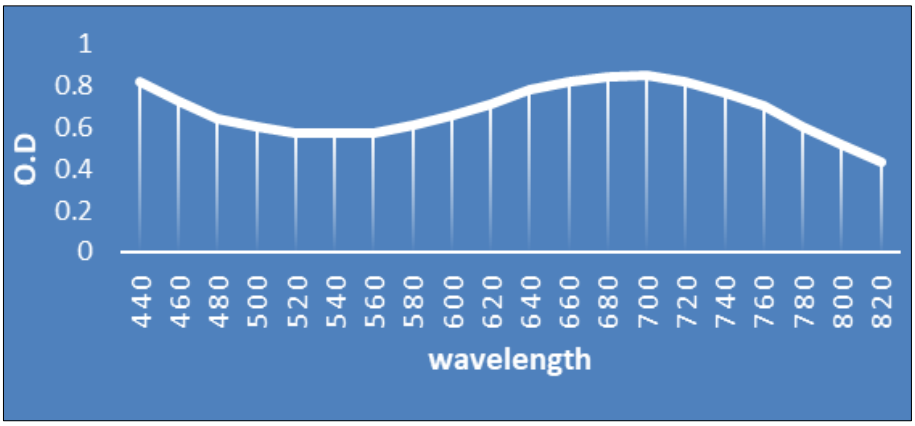


Fig 8: Spectrometric analysis of extracted pigment

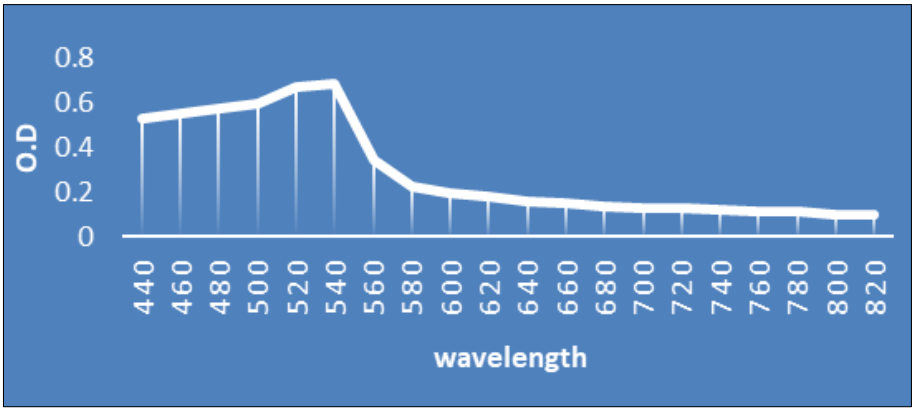


Fig 9: Spectrometric analysis of extracted pigment

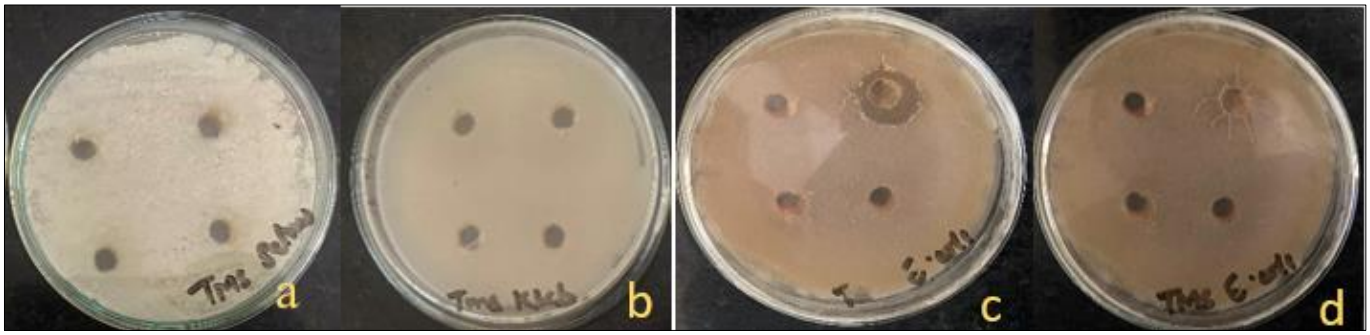
3.8 Antimicrobial activity of pigment extracts of potential isolates against human pathogens:

The antibacterial activity of the four extracted pigments was evaluated against Salmonella typhi (MTCC 98), Pseudomonas aeruginosa (MTCC74), Klebsiella pneumonia (KS19), and E. coli using the well diffusion method and Mueller Hinton agar. Results were observed by measuring the zone of inhibition (Table 4).

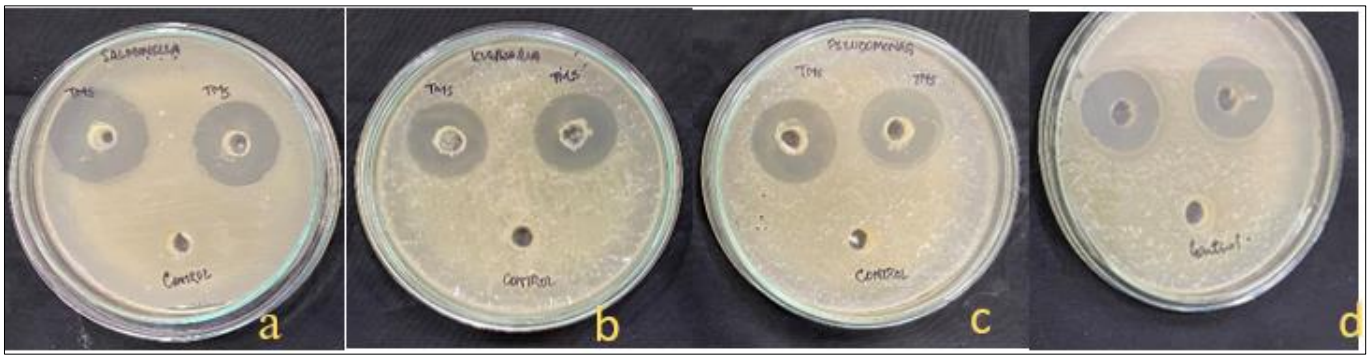
Table 4: Antimicrobial activity of pigment extracts of potential isolates against human pathogens

S. No	Pigment	*Salmonella typhi*	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*	*E.coli*
1	SK-1	0.00±0.00	0.00±0.00	10.00±1.00	0.00±0.00
2	SK-2	12.06±1.98	11.00±1.00	11.03±1.01	10.00±1.00
3	SK-3	12.03±1.01	12.00±2.00	14.00±3.00	11.03±1.01
4	SK-4	12.06±1.98	13.03±2.02	12.06±1.98	10.00±3.00

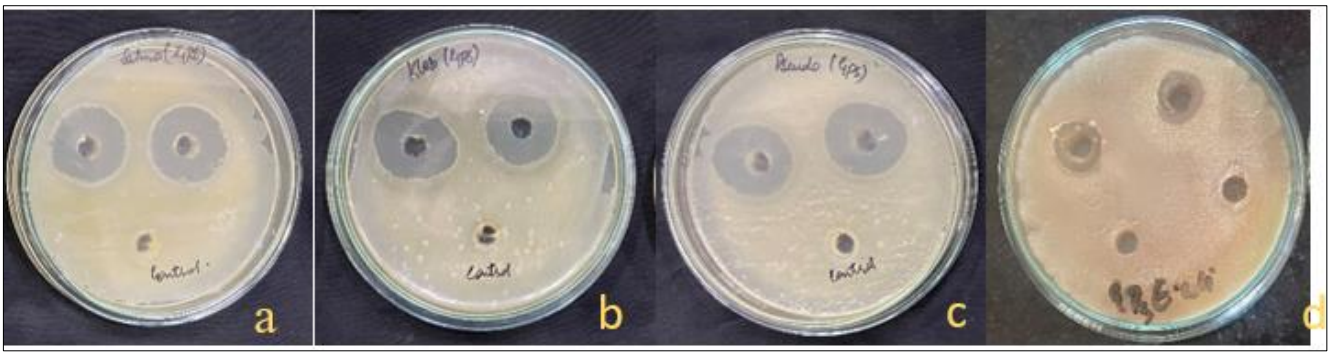
Information presented as Mean Standard Deviation (n=3)



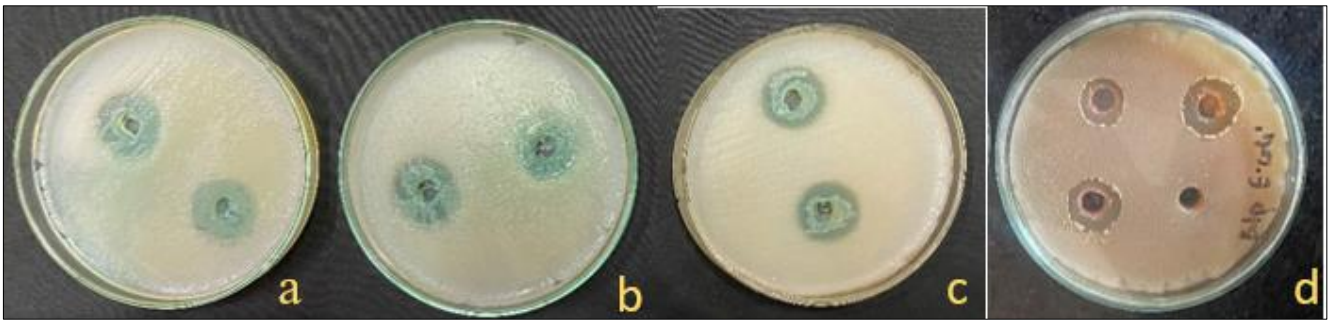
Antimicrobial activity of SK1 against human pathogens where, a is against *Salmonella thypi*, b is against *Klebseilla pneumonia*, c is against *Pseudomonas aeruginosa* and d is against *E.coli*



Antimicrobial activity of SK2 against human pathogens where, a is against *Salmonella thypi*, b is against *Klebseilla pneumonia*, c is against *Pseudomonas aeruginosa* and d is against *E.coli*



Antimicrobial activity of SK3 against human pathogens where, a is against *Salmonella thypi*, b is against *Klebseilla pneumonia*, c is against *Pseudomonas aeruginosa* and d is against *E.coli*



Antimicrobial activity of SK4 against human pathogens where, a is against *Salmonella thypi*, b is against *Klebseilla pneumonia*, c is against *Pseudomonas aeruginosa* and d is against *E.coli*

Fig 10: Antibacterial activity of extracted pigments against different test bacteria

3.9 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Inhibitory Concentration (MIC)

The MIC and MBC values of each pigment extract were determined and the result depicted in the table 5. Showed the MIC value of pigment extracts ranged from 80 to 100 µg/ml, in which for SK1 60 mg/ml *(Pseudomonas aeruginosa)* and *(Klebsiella pneumonia)* showed MIC and its corresponding MBC value ranged 80mµg/ml. Similarly for all the four pigment extracts strongest MIC value ranged between 60-100µg/ml against all the four pathogenic cultures such as K. *pneumonia*, *P. aeruginosa*, S. *typhi* and *E.coli.* A 100-µl aliquot of all tubes with no visible bacterial growth was seeded on MHA plates. Lowest concentration of samples that kills the initial bacterial inoculum is defined as MBC. (Table 5).

Table 5: MIC and MIC of pigment extracts

Pathogenic strains	MIC and MBC (mg/ml)							
	SK1		SK2		SK3		SK4	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Klebsiella pneumonia	60	80	80	100	100	100	100	100
Pseudomonas aeruginosa	60	80	100	100	60	80	80	100
Salmonella typhi	80	100	60	80	60	80	100	100
E.coli	100	100	100	100	100	100	100	100

4. Discussions

Nine pigment-producing bacteria that were light yellow, dark yellow, light orange, reddish pink, and greenish in hue were found and characterised in the current study. The materials

were smeared onto nutrient agar plates and left to incubate for 48 hours at 37 °C. Similar to our studies, Sasidharan *et al*. (2013) [19] and Goswami *et al*. (2010) [8] isolated pigment-producing bacteria from soil and air and cultured them on Nutrient Agar medium.

The maximum absorption of the six isolates of pigment was seen between 440 and 500 nanometers, indicating the presence of carotenoids, while the maximum absorption of the two isolates of green pigment was seen between 550 and 680 nanometers, indicating the presence of phycocyanin. It has been determined that the red pigment's maximum absorption peak corresponds to Prodigiosin's maximum absorption peak, which was shown to occur at 520 and 540 nanometers. According to Goswami *et al*. (2010) [8], Khanafari *et al*. (2010) [9], and Sasidharan *et al*. (2013) [19], carotenoids absorb light between 400 nm and 550 nm. Maximum pigment production was reported by Grossart *et al*. (2009) [7] using Luria Bertani medium. In contrast to other media, Lubria Bertani medium demonstrated good development in the isolates M1 and MS2. After an incubation period of 48–72 hours, bacterial isolates produced good growth and pigment, which then began to decline after 96 hours. On additional incubation, growth and pigment synthesis were reduced (Goswami *et al*., 2010) [8]. In addition, *Salinicoccus* sp. produced orange pigment after a 72-hour incubation period, according to Bhat and Marar (2015) [3].

Antimicrobial activity of all the four extracted pigments was checked against Salmonella typhi (MTCC98), *Pseudomonas* aeruginosa (MTCC74), Klebsiella pneumonia (KS19) and *E.coli*, by well diffusion method using Mueller Hinton agar in which SK1 showed zone of 10mm against *P. aeruginosa* and SK2, SK3 and SK4 showed zones ranging from 10mm-14mm against all the four pathogenic strains such as *P. aeruginosa*, K. pneumonia, *S.typhi* and *E.coli*. Similar results were found by Umadevi and Krishnaveni [21], Manimala and Murugesan [13], and others for carotenoid pigment extracted from Micrococcus luteus KF532949 and *Sporobolomyces* sp. isolated from natural sources, respectively. These researchers reported that Gram-negative bacteria had higher resistance to the carotenoid pigments. The lowest MIC and MBC of 2.8% norbixin for *B. cereus* were also noted by Galindo-Cuspinera *et al*. [6]; additionally, they noted that 2.8% norbixin had no bactericidal effect on *S. typhimurium*. Prodigiosin was isolated from *Serratia marcescens* UFPEDA 398 by Lapenda *et al*. [12] and was found to have greater antibacterial action against Gram + ve bacteria than against Gram -ve bacteria.

5. Conclusion

The current study was done to show the existence of several bacteria that produce colour in various fruits and vegetables. The findings suggest that microbial pigments are excellent sources of pigments, as the need for biocolors is rising due to the dangers of synthetic colours these days. From several fruits and vegetables, including tomato, beetroot, black grapes, papaya, strawberries, and spinach, nine bacteria that produce colours have been discovered. The colours that could be derived from them included yellow, orange, green, and reds. According to molecular analysis, the pigment SK3, also known as prodigiosin, creates reddish pink hues. Similarly, yellow pigment and orange pigment SK1 and SK2 which produce mostly carotenoids and green pigment was produced by SK4 which produce pigment known as phycocyanin. Most of the pigments also showed good antimicrobial activity against the test cultures *viz*; *Salmonella typhi, Pseudomonas aeruginosa, E. coli and Klebsiella pneumoniae.* Bacterial pigment production is now one of the emerging fields of research to demonstrate its potential for various industrial applications. The goal of the study was to identify and separate pigment-producing microorganisms from various sources. Natural colours can be used as colouring agents in a wide range of products, which makes them a potential replacement for manufactured chemicals that can have harmful side effects.

6. References

1. Anzum N, Khan FI, Hossain MZ, Islam MN, Saha ML. Isolation and identification of pigment producing bacteria from the ratargul swamp forest soil. Journal of Biological Sciences 2022;31(1):1-8.
2. Barth M, Hankinson T, Zhuang H, Sperber F, Doyle MP. Microbiological spoilage of fruits and vegetables food microbiology and food safety. 2009;135-183.
3. Bhat MR, Marar T. Media optimization, extraction and partial characterization of an orange pigment from *Salinicoccus* sp. MKJ 997975. Int Jour of Life Science, Biotech and Pharma Res. 2015;4:85-89.
4. Dahiya P, Purkayastha S. Phytochemical Screening and Antimicrobial Activity of Some Medicinal Plants against Multi-drug Resistant Bacteria from Clinical Isolates. Indian Journal of Pharmaceutical Sciences. 2012;6:443-450. Technol. 1(4):361-365
5. Fatima M, Anuradha K. Isolation, Characterization, and Optimization Studies of Bacterial Pigments. Journal of Pure and Applied Microbiology 2022; 16(2):1039-1048.
6. Galindo-Cuspinera V. Volatile composition and antimicrobial properties of commercial annatto (*Bixa orellana* L) extracts, a natural food colorant, Journal of Food Protection. 2003;66(6):1074-8.
7. Gossart HP, Thorwest M, Plitzko I, Brinkhoff T, Simon M Zeeck A. Production of a blue pigment by marine *Rheinheimera* sp. Int Jour of Microbiol. 2009;47:387-394.
8. Goswami G, Chaudhuri S, Dutta D. Effect of pH and temperature on pigment production from an isolated bacterium. Chem Engin Trans. 2010;20:127-132.
9. Khanafari A, Khavarinejad D, Mashinchian A. Solar salt lake as natural environmental source for extraction halophilic pigment. Iran Jour of Microbiol. 2010;2:103-109.
10. Koes RE, Quattrocchio F, Mol JN. The flavonoid biosynthetic pathway in plants: function and evolution. Bio Essays. 1994;16(2).
11. Kowalska-Krochmal B, Dudek-Wicher R. The minimum inhibitory concentration of antibiotics: Methods, interpretation, clinical relevance. Pathogens. 2021; 10(2):165.
12. Lapenda JC, Silva PA, Vicalvi MC, Sena KX, Nascimento SC. Antimicrobial activity of prodigiosin isolated from Serratia marcescens UFPEDA 398. World J Microbiol Biotechnol. 2015;31(2):399-406.
13. Manimala MRA, Murugesan R. *In vitro* antioxidant and antimicrobial activity of carotenoid pigment extracted from *Sporobolomyces* sp. isolated from natural source. J Appl Natural Sci. 2014;6:649 -53
14. Pankaj VP, Kumar R. Microbial pigment as a potential natural colorant for contributing to mankind, Research

Trends in Molecular Biology; c2016. p. 85-98.
15. Parvekar P, Palaskar J, Metgud S, Maria R, Dutta S. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against Staphylococcus aureus. Biomat Invest Dentistr. 2020;7(1):105-9.
16. Podder K, Padhan B, Sarkar D, Sarkar A Purifcation and optimization of pink pigment produced by newly isolated bacterial strain *Enterobacter* sp. PWN1. SN Applied Sciences. 2020;3:105.
17. Priya M, Selvi CS, Kousalya M Screening for potential carotogenic producing bacteria from rhizospheric soil and it act as an antibacterial activity. World Journal of Pharmacy and Pharmaceutical Sciences. 2017;6(12):1033-1043.
18. Qayyum S, Basharat S, Mian AH, Qayyum S, Ali M, Changsheng P, Shahzad M, Sultan F. Isolation, identifcation and antibacterial study of pigmented bacteria. Applied Nanoscience. 2020;10:4495-4503.
19. Sasidharan P, Raja R, Karthik C, Sharma R, Arulselvi P I. Isolation and characterization of yellow pigment producing *Exiguobacterium* sp. Jour of Biochem Tech. 2013; 4:632-635.
20. Sinha S, Choubey S, Kumar A, Bhosale P. Identification, characterization of pigment producing bacteria from soil and water and testing of antimicrobial activity of bacterial pigments. International Journal of Pharmaceutical Sciences and researches. 2017;42(2):119-124.
21. Umadevi K, Krishnaveni M. Antibacterial activity of pigment produced from Micrococcus luteus KF532949. Int J Chem Anal Sci. 2013;4:149 -52.
22. Waghela M, Khan, S. Isolation, Characterization of Pigment Producing Bacteria from Various Food Samples and Testing of Antimicrobial Activity of Bacterial Pigments. DAV International Journal of Science. 2018;7(1):2277-564.