International Journal of Biology Research
www.biologyjournal.in
ISSN: 2455-6548
Received: 29-03-2025, Accepted: 28-04-2025, Published: 13-05-2025
Volume 10, Issue 2, 2025, Page No. 6-12

Production of melanin from *Bacillus spp.* using different fruit waste

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Abstract
Melanin, a versatile biopolymer with applications in medicine, cosmetics, and food industries, can be sustainably produced using microbial sources. This study investigates the production of melanin by *Bacillus spp*. utilizing different fruit wastes as a cost-effective and eco-friendly substrate. Various fruit wastes, including Orange, Pineapple, Pomegranate waste were assessed for their efficacy in supporting melanin synthesis. The bacterial strains were cultivated on optimized fermentation media, and melanin yield was quantified spectrophotometrically. The results demonstrated that fruit waste serves as a suitable carbon source for melanin production, with variations in yield based on the type of waste used. Characterization of the extracted melanin was performed using UV-Vis spectroscopy and FTIR analysis to confirm its structural properties. This study highlights the potential of utilizing agro-industrial waste for sustainable melanin biosynthesis, offering an environmentally friendly approach to biopolymer production.

Keywords: Melanin, *Bacillus* spp., fruit waste, biopolymer, sustainable

Introduction

Natural pigments are considered safe, with pronounced multifarious benefits compared to synthetic pigments, among those, 'Melanin' forms a ubiquitous heterogeneous polymer group with a broad range of structural and functional diversity (Gosset, 2017; Stepien *et al.,* 2013) [5, 13]. Melanins are the natural pigments which have their presence in animals, plants and in most of the microorganisms. They are the dark coloured negatively charged high molecular weight pigments which are formed due to polymerized phenolic and/or indolic compounds. These complex polymers are amorphous in nature and shows solubility in neither aqueous nor organic solvents. They showed resistance to concentrated acids and are susceptible to bleaching by oxidizing agents They play a vital role in defence and protection mechanisms that Improve the survival and competitive- ness of the organism. Melanin is known for it's absorption capacity of radiation of all wavelengths with an optimum absorbance at UV range which prevents photo induced damage. Hence it is used in the preparation of photo absorbing optical lenses and in bioplastics. Besides photo protection it has versatile biological activities such as radical scavenging, antioxidant, antitumor, anti- inflammatory and as immune stimulating agent.

Melanin has long been an important component of living organisms and cells. Melanin synthesis in organisms is primarily involved in the protection of host cells and organisms. This includes protection from UV radiation and energy absorption, protection from external physical changes, and maintenance of intracellular homeostasis through its physiological activity (Bolognese *et al.,* 2019; Seo and Choi, 2020) [4, 10]. The phenomenon disrupts the important processes like Genomic replication, Transcription, and Translation. For avoiding the DNA damage and other harms by the UV and above spectrums of light, the organisms are prone to secrete or produce a chemical or a pigment that has the ability to absorb the Ultraviolet light. This chemical is known to be as 'Melanin.

Melanin and its different types are the natural pigments which have their presence in all the five kingdoms of the living organisms. The compound melanin is predominantly Indolic in its nature and is found to be abundantly produced on the surface of the vertebrates, such as their skins. In the higher organisms this pigment is produced in specialized cells called the Melanocytes. The process for the formation of the Melanin is called as Melanogenesis, this process is generally triggered by the exposure of the melanocytes to the ultraviolet light. This pigment is also found to be secreted in microorganisms in their cytoplasmic organelles called the Melanosomes specifically. The origin of this word which is Melanin comes from Ancient Greek word called melons which means dark in the mentioned language. Based on the Colour and the structural orientation of the atoms of the compound melanin, it can be broadly classified in three major classes which are: Eumelanin, Pheomelanin, and Allomelanin (Pathan and Pethe 2016; Simon *et al.,* 2001) [8, 11].

Melanin obtained from microbes has great advantages over melanin from animals and plants. Microorganism don't cause the problems of seasonal variations and are selected arsenals as they modify them according to the medium and conditions provided to the Targeting melanogenisis in microbes may help to discover antimicrobial drugs. For example, melanins produced by *Cryptococus* by *Cryneoformans* and *Burkholdericcepacia* offer virulence and contribute to the growing resistance of these pathogenic bacteria towards antibiotics. The melanin synthesized by microbes shows metal chelating ability to sorb the radioactive (wastes uranium). There are reports that showed the anti HIV properties of melanin and their role in photo voltage generation and fluorescence studies. Therefore, all these properties of melanin make them unique and are widely used in cosmetic, sunscreen protection creams, eye glasses, pharmaceuticals, and food industries (Tarangini & Mishra, 2014) [14]

Material and methods

Collection of Fruit Waste:

Fruit waste was obtained from a local fruit juice shop located on Gandi Road, near the bus stand and GMC Road Akola. The waste consisted of remnants from oranges, pineapples, and pomegranates, including extracted pulp, cores, crushed seeds, and arils. The fruit waste was transported in sterile, autoclaved polythene bags for further analysis.

Preparation of media for melanin production:

- **Fruit waste medium**: Take 250 grams of fruit waste and add 500 milliliters of distilled water. Heat the mixture to 100°C and boil for 30 minutes. After boiling, filter the mixture through muslin cloth to separate the liquid from the solid waste. Store the filtered liquid at 4°C for further used.
- **Preparation of tyrosine enriched fruit waste medium:** Take 100 ml of the prepared fruit waste medium. Add 1 gm of tyrosine to the medium boil the mixture at 100°C for 10 minutes to ensure the tyrosine dissolves completely allow the medium to cool at room temperature Inoculate the cooled medium with 1ml of melanin producing isolate (NT) Incubate the inoculated medium at 37°C for 15 days to allow for melanin production.
- **Tyrosine basal broth**: 1 test tubes containing 10ml L-tyrosine broths were inoculated in with the isolated bacterial isolates. Later the 10ml test tubes after incubation of 24 Hour at 37°C were subjected to melanin production in 250ml flasks containing 100ml L-tyrosine broth. The 100ml inoculum of the bacterial strains was later subjected to 1000ml flasks containing TBB media (Tyrosine Basal Broth) for the production of melanin. After the successful incubation of 15 days at 37°C, the 10ml sample from each flask was centrifuged and read spectrophotometrically at 400nm for the estimation of the concentration of melanin produced (Jigna *et al.,* 2022; Turick *et al.,*2002) [6]. This broth is use as a control.

Production of melanin by isolate:

Melanin-producing bacteria were isolated from the rhizosphere of Neelgiri trees using L-tyrosine agar. The isolates were sub-cultured and grown in nutrient broth before inoculation into TBB and Fruit Waste Medium. After 24 hours of incubation at 37°C, a dark pigment was observed. The medium was centrifuged to separate cells, and melanin was extracted by acidifying the supernatant (pH 2) and allowing precipitation for 48 hours. The precipitate was collected, washed with alcohol and water, dried, and weighed.

Extraction and purification of melanin:

After 15 days of incubation, the TBB and fruit waste medium were centrifuged at 6000 rpm for 10 minutes at 28°C to separate bacterial pellets. The supernatant was acidified to pH 2 using 1 N HCl, leading to black precipitate formation. After 2 days of precipitation, the melanin was pelleted by centrifugation, washed twice with ethanol and deionized water, and dried in a hot air oven for purification.

Solubility test of the extracted melanin:

For testing the solubility of the extracted melanin 40 test tubes were used in the set of 10 tubes for each extracted melanin pigment. Each set of test tubes had 1 ml of the following compounds: Hydrochloric acid (HCI), Petroleum ether, Chloroform, Hydrogen peroxide (H2O2, Acetic acid, Sodium hydroxide (NaOH), Dimethyl sulfoxide (DMSO), Iso-propanol, Ethanol and Distilled Water. The extracted melanin was added in the test tubes and then the tubes were vortexed in the vortex machine for 5 min, then centrifuged at 5000rpm for 1 min. The test tubes were observed for solubility of melanin (Fava *et al.,* 2013).

Determination of absorption spectrum of melanin by UV-Visible spectroscopy:

The melanin pigments were read spectrophotometrically using the melanin solution at 200nm to 800nm at the interval gaps of 50nm (wavelength of the electromagnetic spectrum) in a UV-Visible spectrophotometer. Results were noted for further study.

Fourier-transform infra-red (ft-ir) spectroscopy:

The FT-IR spectrum was recorded at the wavelength of 4000-400 cm-1. Characteristic peaks were obtained and the recorded report was analyzed. The extracted melanin samples were mixed with potassium bromide and grinded in a mortar and pestle, the melanin-KBr pellet was put in a laboratory hydraulic press to convert the pellet into discs, the discs were set into the FT-IR machine and then IR-radiations were bombarded on to the sample, of 4000-400 cm-1 wavelength to make the molecule of the sample to vibrate and on the basis of the molecular vibrations and molecular stretching of the functional groups we get the IR-spectra with characteristic peaks of different functional groups of the molecules.

Results and discussion

The morphological, cultural and biochemical characteristics of melanin producing bacterial isolate NT which was isolated from the rhizosphere of Neelgiri trees was determined using different morphological, cultural, and biochemical methods (Table1). Different tests performed confirmed that the bacterial isolates NT belongs to *Bacillus spp.*

The bacterial isolate (NT) was inoculated into 1000 mL of Tyrosine Basal Broth (TBB) and separately into Tyrosine-Enriched Pineapple Waste Medium, Tyrosine-Enriched Orange Waste Medium, and Tyrosine-Enriched Pomegranate Waste Medium. These cultures were incubated at 37°C for 15 days. After the Incubation period, the media in all conical flasks turned completely dark, indicating Melanin production. The cultures were then centrifuged at 6000 rpm at 28°C for 10 Minutes to separate the bacterial pellets from the melanin-containing supernatants. The supernatants were acidified using 1N HCl until the pH dropped below a certain Level, leading to melanin precipitation at the bottom of the flasks. After two days, the Precipitates were centrifuged again at 6000 rpm at 28°C for 10 minutes to collect the Melanin. The extracted melanin pigments were washed twice using absolute ethanol and deionized water to obtain purified melanin. The yield of purified melanin varied across the different media. Tyrosine Basal Broth media (control) gave 1.5 gm of Purified melanin, Tyrosine-Enriched Pineapple Waste media gave 0.7 gm of purified Melanin, Tyrosine-Enriched Orange Waste media gave 1.0 gm of purified melanin and The Tyrosine-Enriched Pomegranate Waste media gave 1.2 gm of partially Purified melanin.

The solubility of the extracted melanin pigments from the bacterial Isolate was tested. It was found that the melanin pigment extracted from NT isolate (Control) was soluble in Petroleum ether, Acetic acid, Sodium hydroxide (NaOH) and Dimethyl sulfoxide (DMSO). The pigment was found to be insoluble in Hydrochloric Acid (HCl), Chloroform, Ethanol, Water and Iso-propanol. While it was found that the Pigment was partially soluble in Hydrogen peroxide (H2O2). The extracted melanin pigment from Pineapple fruit waste was found to be soluble in Petroleum ether, Acetic acid, Hydrogen peroxide and Dimethyl sulfoxide (DMSO). The pigment was found to be insoluble in Hydrochloric acid (HCl), Chloroform, Ethanol, Water and Iso-propanol. While it was found that the pigment was partially soluble in Hydrogen Peroxide (H2O2). The extracted melanin pigment from Orange waste was found to be Soluble in Petroleum ether, Acetic acid, Sodium hydroxide (NaOH), Dimethyl Sulfoxide (DMSO). The pigment was found to be insoluble in Hydrochloric acid (HCl), Chloroform, Ethanol, Water and Iso-propanol. While it was found that the pigment was partially soluble in Hydrogen peroxide (H2O2). The extracted melanin pigment from Pomegranate was found to be soluble in Petroleum ether, Ethanol, Acetic acid, Sodium hydroxide (NaOH), Dimethyl sulfoxide (DMSO). The pigment was found to be insoluble in Hydrochloric acid (HCl), Chloroform, Water. While it was found to that the pigment was partially soluble in Hydrogen peroxide (H2O2), Isopropanol.

Table 1: Determination of Solubility of extracted melanin from the bacterial isolate

Sr. No	Solvent	Solubility			
		Control (NT)	Pineapple	Orange	Pomegranate
1	Hydrochloric acid	Insoluble	Insoluble	Insoluble	Insoluble
2	Petroleum ether	Soluble	Soluble	Soluble	Soluble
3	Chloroform	Insoluble	Insoluble	Insoluble	Insoluble
4	Ethanol	Insoluble	Insoluble	Insoluble	Soluble
5	Water	Insoluble	Insoluble	Insoluble	Insoluble
6	Hydrogen peroxide	Partially soluble	Soluble	Partially soluble	Soluble
7	Acetic acid	Soluble	Soluble	Soluble	Soluble
8	Sodium hydroxide	Soluble	Soluble	Soluble	Soluble
9	Dimethyl sulfoxide	Soluble	Soluble	Soluble	Soluble
10	Iso-propanol	Insoluble	Insoluble	Insoluble	Partially soluble

The UV-Visible spectroscopy of the extracted melanin pigments was performed. The melanin pigment isolated from NT isolate (Control) showed the maximum absorbance of 1.734 at 200nm followed by 1.247 at 250nm, 1.242 at 300nm, 1.240 at 350 nm, 1.100 at 400nm, 0.948 at 450nm, 0,676 at 500nm, 0.394 at 550nm, 0.237 at 600nm, 0.085 at 650nm, -0.110 at 700nm, -0.119 at 750 nm and 0.119 at 800 nm. The melanin pigment extracted from Pineapple waste showed maximum absorbance of 1.245 at 200nm followed by 1.198 at 250nm, 1.186 at 300nm, 1.175 at 350nm, 1.098 at 400nm, 0.986 at 450nm, 0.747 at 500nm, 0.222 at 550nm, 0.1 12 at 600nm, 0.076 at 650nm, -0.112 at 700nm, -0.122 at 750nm, -0.122 at 800nm. The melanin pigment extracted from Orange waste showed maximum absorbance of 1.347 at 200nm followed by 1.290 at 250nm, 1.200 at 300nm, 1.198 at 350, 1.075 at 400nm, 0.992at 450mm, 0.721 at 500nm, 0.328 at 550, 0.128 at 600nm, 0.082 at 650nm, -0.118 at 700nm, -0.130 at 750nm, -0.130 at 800nm. The melanin pigment extracted from Pomegranate waste showed maximum absorbance of 1.437 at 200nm followed by 1.367 at 250nm, 1.307 at 300nm, 1.208 at 350nm, 1.031 at 400nm, 0.921 at 450, 0.917 at 500nm, 0.776 at 500nm, 0.569 at 600nm, 0.250 at 650nm, -0.126 at 700nm, -0.137 at 750 and -0.137 at 800nm. The graph was plot considering absorbance at particular wavelength of melanin (Table 2).

Table 2: Characterization of extracted melanin from bacterial isolate by UV-Visible Spectroscopy

Sr. No	Wavelength (nm)	Absorbance (O.D)			
		Control (NT)	Pineapple	Orange	Pomegranate
1	200	1.734	1.245	1.347	1.437
2	250	1.247	1.198	1.290	1.367
3	300	1.242	1.186	1.200	1.307
4	350	1.240	1.175	1.198	1.208
5	400	1.100	1.098	1.075	1.031
6	450	0.948	0.986	0.992	0.921
7	500	0.676	0.747	0.721	0.917
8	550	0.394	0.222	0.328	0.776
9	600	0.237	0.112	0.128	0.569
10	650	0.085	0.076	0.082	0.250
11	700	-0.110	-0.112	-0.118	-0.126
12	750	-0.119	-0.122	-0.130	-0.137
13	800	-0.119	-0.122	-0.130	-0.137

Production of melanin from Different sources. Control Sample (Standard Melanin Production). The control sample produced 1.5 g/L of melanin production. This value serves as the baseline for comparison. When pineapple was used, melanin production dropped to 0.7 g/L. This is a significant decrease of 0.8 g/L (53.3%) compared to the control

Pineapple showed the lowest melanin production among all tested sources. The use of orange waste resulted in 1.0 g/L of melanin production. Compared to the control, this is a reduction of 0.5 g/L (33.3%). However, orange performed better than pineapple in melanin production. Pomegranate waste led to 1.2 g/L of melanin production. This is only 0.3g/L (20%) lower than the control, indicating a moderate reduction. All fruit sources resulted in lower melanin production compared to the control, with pomegranate showing the highest production among the three fruits waste, followed by orange and then pineapple. A graph was plotted to visualize melanin production using different fruit waste (Figure 1).

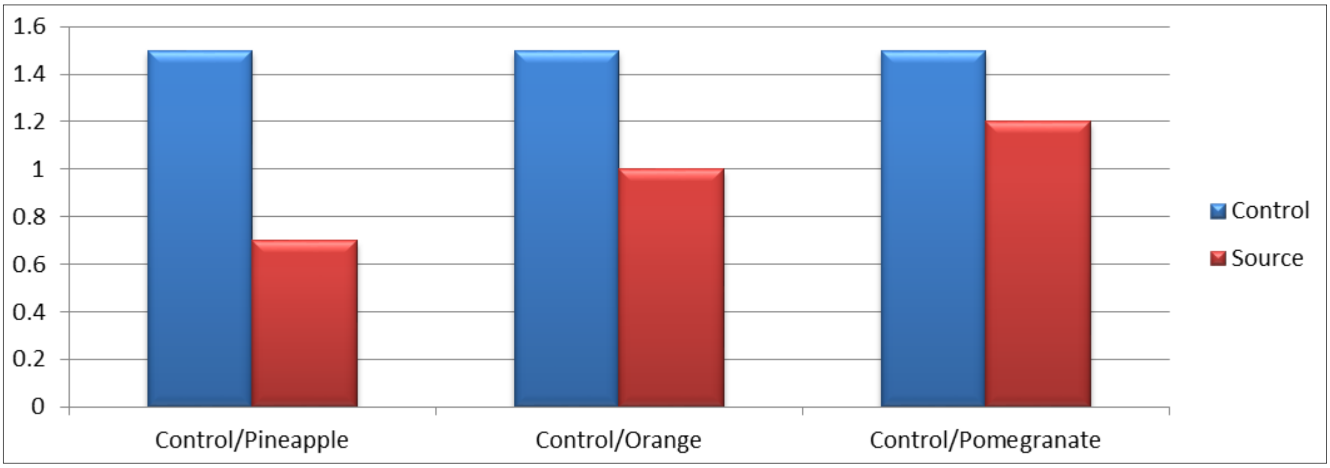


Fig 1: Production of melanin using different fruit waste

For further characterization of the pigment produced by the bacterial isolates was indeed melanin, FT-IR analysis of the pigments was performed. For this analysis IRAffinity-1 spectrophotometer from SHIMADZU was used. The melanin pigments from the isolates were mixed with KBr (Potassium bromide) and grinded with mortar and pestle. The formed pellet was pressed in a laboratory hydraulic press converting the pellets into discs. These discs were then loaded into the FR-IR spectrophotometer. The IR spectra and the data table was obtained for the respective melanin pigments. The control melanin pigment showed peak at 3400.65 which was broad peak which is For -OH (acid group stretching), -COOH stretching and a potential -NH stretching. A peak at wavelength 2930.00 confirmed the presence of -CH stretching and another Peak at 1639.56confirms the presence of vibration of aromatic C-C group, and -C-O Stretching (Figure 3).The Pineapple waste melanin pigment showed a broad Peak at 3412.22 which confirms the presence of -NH. Broad peak corresponding to –NH group at 2930.96 confirms the presence of aliphatic -CH stretching with some association with the aromatic C-C and -C-O stretching confirming with the peak at 1642.46 (Figure 4). The Orange waste melanin pigment showed a broad peak at 3401.61 and 3304.20 which confirms the presence of -NH. Broad peak corresponding to -NH group at 2930.96 confirms the presence of aliphatic -CH stretching with some association with the aromatic C-C and -C-O stretching confirming with the peak at 1642.46 (Figure 5). The Pomegranate waste Pigment showed peak at 2891.42 which was a broad peak which is for -OH (acid group stretching), -COOH stretching and a potential -NH stretching. A peak at 1657.89 wave length confirmed the presence of -CH stretching and another peak at 1112.97 confirms the presence of vibration of aromatic C-C group, and -C-O Stretching (Figure 6). The presence of the functional groups like -NH, -COOH, CH, C=C, C=O, -O-H (acid group) confirms the pigments may belongs to be melanin.

Discussion

In the present study, a *Bacillus species* capable of melanin production was selected. Similar findings were reported by Barate and Dange (2024) [3], where they documented melanin production in *Bacillus species* isolated from the rhizosphere soil of Neelgiri.

The current study dealt with the melanin pigments isolated from the bacterial Isolate. The melanin from these isolate was extracted and partially purified. It was found that the control produced a higher quantity of melanin compared to the Pineapple waste, orange waste, and pomegranate waste. The extracted melanin was characterized, in which solubility test was performed for the extracted melanin for checking the chemical properties. In the present study it was found that It was found that the melanin pigment extracted from NT isolate (Control) was soluble in Petroleum ether, Acetic acid, Sodium hydroxide (NaOH) and Dimethyl sulfoxide (DMSO). The pigment was found to be insoluble in Hydrochloric acid (HCL), Chloroform, Ethanol, Water and Iso-propanol. While it was found that the pigment was partially soluble in Hydrogen peroxide(H_2O_2) . The extracted melanin pigment from Pineapple fruit waste was found to be soluble in Petroleum ether, Acetic acid, Hydrogen peroxide and Dimethyl sulfoxide (DMSO). The pigment was found to be insoluble in Hydrochloric acid (HCI), Chloroform, Ethanol, Water and Iso-propanol. While it was found that the pigment was partially soluble in Hydrogen peroxide (H_2O_2). The extracted melanin pigment from Orange waste was found to be soluble in Petroleum ether, Acetic acid, Sodium hydroxide (NaOH), Dimethyl sulfoxide (DMSO). The pigment was found to be insoluble in Hydrochloric acid (HCI), Chloroform, Ethanol, Water and Iso-propanol. While it was found that the pigment was partially soluble in Hydrogen peroxide (H_2O_2). The extracted melanin pigment from Pomegranate was found to be soluble in Petroleum ether, Ethanol, Acetic acid, Sodium hydroxide (NaOH), Dimethyl sulfoxide (DMSO). The pigment was found to be insoluble in Hydrochloric acid (HCI), Chloroform, Water. While it was found to That the pigment was partially soluble in Hydrogen peroxide(H_2O_2), Iso-propanol.Study conducted by Jigna *et al.,* (2022) [6] reported that melanin pigment produced by *Bacillus pumilius* isolated from sea water sample was soluble in NaOH, while Insoluble in water, ethanol, methanol, iso-propanol, acetic acid, HCl and sparingly Soluble in DMSO. Likewise, Tarangini and Mishra (2014) [14] reported melanin produced from *Pseudomonas spp* found to be insoluble in water, ethanol, chloroform, acetone and benzene and slightly soluble in phenol and NaOH. Arun *et al.,* reported that Melanin produced by *Schizophyllum commune* (Fries) found to be insoluble in water and organic solvents such as ethanol, benzene, hexane, Petroleum ether or acetone but Soluble in KOH or NaOH.

In the current study, the production of melanin from different sources was evaluated. The control sample, representing standard melanin production, yielded 1.5 g/L. This value serves as the baseline for comparison. When pineapple was used, melanin production dropped significantly to 0.7 g/L, a decrease of 0.8 g/L (53.3%) compared to the control. Pineapple resulted in the lowest melanin production among all tested sources. Orange waste led to a melanin production of 1.0 g/L, which is 0.5 g/L (33.3%) lower than the control. While this represents a reduction, orange performed better than pineapple in terms of melanin production. Pomegranate waste yielded 1.2 g/L of melanin, showing a moderate reduction of 0.3 g/L (20%) compared to the control. Study by Ravi *et al.*

., (2020) Investigated melanin production using fruit peels and found that pomegranate waste yielded higher melanin content compared to citrus peels. This aligns with the current study, where pomegranate waste produced the highest melanin among the tested fruit sources. Tarangini and Mishra (2014) [14] Examined microbial melanin synthesis using various fruit residues and reported that Orange waste provided a moderate yield of melanin. Singh and Patel (2021) Research Demonstrated that melanin production is highly dependent on the nutrient composition of the substrate. It was observed that pineapple waste had lower nitrogen content, which could be a reason for reduced melanin production.

In the current study, to find out the maximum absorbance of the melanin Pigments, UV-Visible spectroscopy was performed. It was found that the melanin Extracted from isolate Control (NT) showed maximum absorbance of 1.734 at 200nm. The melanin extracted from the Pineapple waste showed maximum absorbance of 1.245 at 200nm. The melanin extracted from Orange waste showed maximum absorbance of 1.347 at 200nm, and the melanin extracted from Pomegranate showed Maximum absorbance of 1.437 at 200nm. As the Wavelength of light increased towards the visible spectrum the melanin pigments showed a steep drop in the Absorbance and the above the Wavelength of 750 nm and 800nm the melanin Pigments showed the same absorbance. Study conducted by Elsauis *et al.,* (2022) Reported that the melanin pigment produced by Hortaeawerneckii showed the Maximum absorbance at the Wavelength of 240nm, similar pattern in the current Study of decrease in absorbance as the wavelength reaches visible spectrum is seen in This study. Tarangini and Mishra, (2014) [14]. Reported the melanin produced using fruit Waste extract also show the maximum absorbance around UV spectrum of 230nm.

In the current study FT-IR spectroscopy was utilizes for further characterization of melanin pigments. The melanin pigments isolated from bacterial Isolate showed presence of -NH, -OH (acidic), -COOH -CH and possible C =C (aromatic) stretching, which was interpretated from the spectra and the data obtained from the FT-IR. The control melanin pigment showed peak at 3400.65 which was Broad peak which is for-O-H (acid group stretching), -COOH stretching and a Potential -NH stretching. A peak at wavelength 2930.00 confirmed the presence of –CH stretching and another peak at 1639.56 confirms the presence of vibration of Aromatic C-C group, and -C-O stretching (Figure 2). The Pineapple Waste Melanin pigment showed a broad peak at 3412.22 which confirms the presence of -NH. Broad peak corresponding to -NH group at 2930.96 confirms the presence of Aliphatic -CH stretching with some association with the aromatic C-C and-C-O Stretching confirming with the peak at 1642.46 (Figure 3). The Orange waste Melanin pigment showed a broad peak at 3401.61 and 3304.20 which confirms the Presence of -NH. Broad peak corresponding to -NH group at 2930.96 confirms the Presence of aliphatic-CH stretching with some association with the aromatic C=C And-C-O stretching confirming with the peak at 164246 (Figure 4). The Pomegranate waste pigment showed peak at 2891.42 which was a broad peak which is for-O-H (acid group stretching), -COOH stretching and a potential-NH stretching. A peak at 1657.89 wavelength confirmed the presence of -CH stretching and another Peak at 1112.97 confirms the presence of vibration of aromatic C-C group, and-C-O Stretching (Figure 5). The presence of the functional groups like -NH, -COOH, CH, C=C, C=O, -O-H (acid group) confirms the pigments indeed to be Melanin. Liu *et al.,* (2022) studied fungal melanin and found characteristic peaks around 3400 cm-1(-OH, -NH), 2920 cm-1(-CH stretching), and 1625 cm-1(aromatic C=C, C=O stretching), which are similar to the observed spectra in the present study. Pralea *et al.,* (2019) [9] confirmed that bacterial and plant-derived melanin exhibit broad Peaks in the 3300–3500 cm-1 Region, correlating to -NH and -OH groups, and peaks Between 1600–1650 cm-1, corresponding to aromatic vibrations. Rashid et al., (2021) investigated melanin from different microbial sources, revealing peaks in the range of 1600–1650 cm-1, indicative of aromatic ring stretching, and aliphatic CH stretching around 2900 cm-1, further corroborating the findings in this study. Tarangini and Mishra (2014) [14] reported the FT-IR report and spectrum of melanin which was extracted from *Pseudomonas spp,* show the absorption peak at 3373 indicating the Presence of -OH and -NH stretching and the sharp peak of 1650 confirms the presence of C-C aromatic carbons.

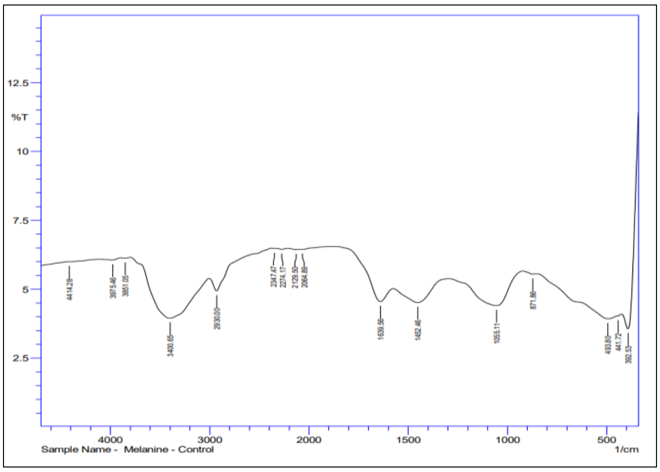


Fig 2: FT-IR Spectra of melanin from NT bacterial Isolate (Control)

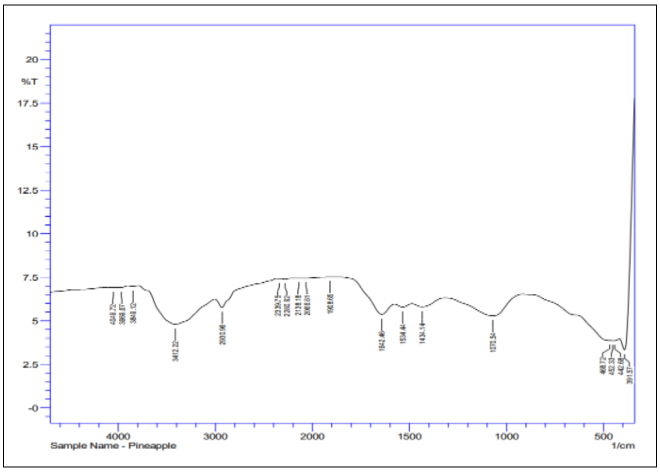


Fig 3: FTIR spectra of melanin from pineapple waste

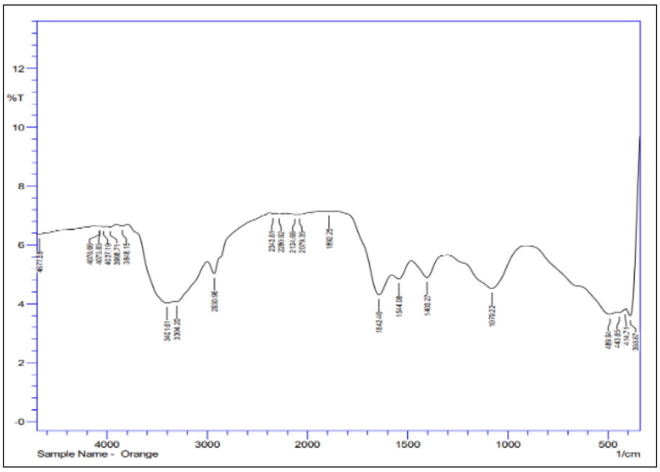


Fig 4: FTIR spectra of melanin from orange waste

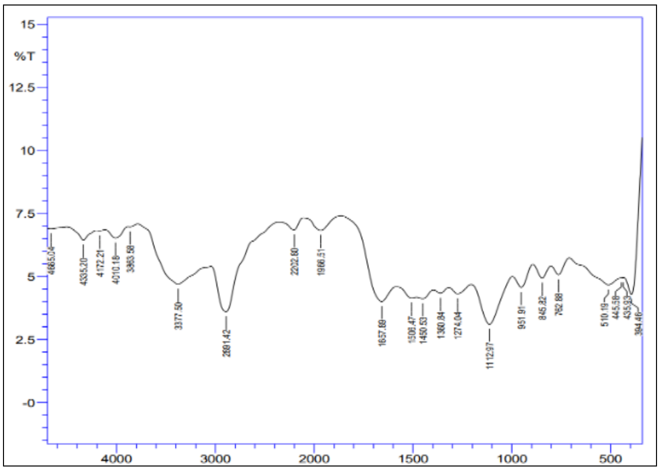
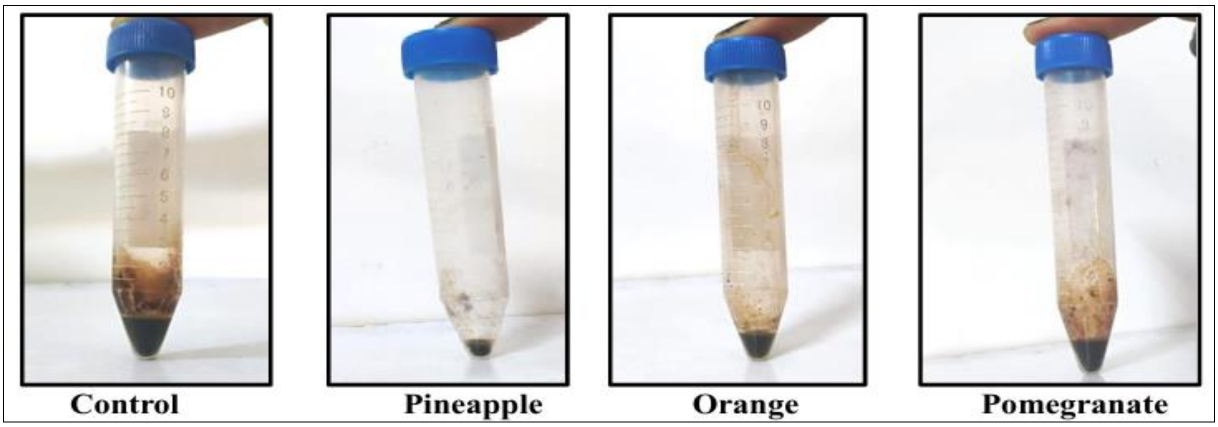


Fig 5: FTIR spectra of melanin from pomogranate waste

Production of melanin using different fruit waste



Extracted melanin from isolate using different fruit waste

Conclusion

In the present study a melanin-producing *Bacillus species* from the rhizosphere of Neelgiri tree was utilized for the production of melanin. The bacterial Isolate (NT) produced melanin in Tyrosine Basal Broth and fruit waste-enriched Media, with the highest yield observed in the control. Among the fruit wastes, Pomegranate waste resulted in the highest melanin production followed by orange and Pineapple. The extracted melanin pigments were analyzed for solubility, UV-Visible Spectroscopy, and FT-IR spectroscopy. The solubility tests confirmed variations in Solvent compatibility, while UV-Visible spectroscopy revealed peak absorbance Around 200 nm, followed by a gradual decline towards the visible spectrum. FT-IR Analysis confirmed the presence of key functional groups (-NH, -OH, -COOH, C=C, C-O), validating the pigments as melanin. These findings highlight the potential of *Bacillus spp*. The present study reveals melanin biosynthesis different organic Substrates influence yield and properties. The study contributes to the understanding of microbial melanin production and its applications in biotechnology using cheap Raw sources and also helpful in controlling environment pollution due to the generated fruit waste.

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