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Bio-production and characterization of carotenoid yellow pigment from *Kocuria* sp. GMA and exploring its sustainable antioxidant, antimicrobial and antibiofilm properties

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Abstract

Bacterial pigments particularly carotenoid provide interesting prospects for a range of applications as antioxidants, antibacterial, and food additives. In-addition they are regarded as a competitive substitute for natural color production due to their better biodegradability and higher compatibility with the environment. This study aims to investigate the processes involved in producing carotenoid yellow pigment (CYP), including strain isolation and identification, pigment extraction, chemical characterization of the pigment, and testing of the pigment's antimicrobial, antioxidant, and antibiofilm properties. Using morphological traits and 16S rRNA sequencing, the isolate was recognized as *Kocuria sp*. GMA, with accession number OM921388. CYP from *Kocuria sp* .first, FTIR, LC-ESI-MS/MS spectrophotometers, and UV absorption spectra were used to chemically characterize CYP from Kocuria sp. GMA. In the pigment extract of *Kocuriasp.* bacteria, seven compounds, mostly carotenoids, were identified in the obtained LC-MS spectra: 224.04, 536.05, 553.22, 540.17, and 704.27, which correspond to a molecular weight of 224, 536, 552, 538.9, and 704 g/mol, which refer to Kocumarin, Lycopene, beta-cryptoxanthin, Neurosporene, and Flavuxanthin, respectively; peaks at m/z [M-H] lead to a molecular weight of 705.67 and 223.06, which refer to Kocumarin and Sarcinaxanthin, respectively. CYP possesses highly antioxidant activity (95.6%); this activity increased gradually with increasing concentrations and time, with an IC50 of 4.0 mg/ml at 90 min and 6.0 mg/ml at 60 min. Also, CYP showed moderate antimicrobial activity against the test pathogens with different concentrations, while it showed excellent activity as an antifungal against *Aspergillus niger* NRRLA-326. The MIC and MBC values of CYP against Gram-positive and Gram-negative bacteria ranged from 10 to 50 µg/ml. Results showed great antibiofilm activity of CYP against *S. aureus* NRRLB-767 and moderate activity against *E. Coli* ATCC 25922.

Keywords: *Kocuriasp*., Carotenoid Yellow Pigment, Chemical Characterization, Antioxidant, Antimicrobial and Antibiofilm activity

1. Introduction

There is an increasing level of interest in microbial pigments owing to their inherent natural characteristics and safety for use. These pigments possess therapeutic benefits and are rich in essential nutrients such as vitamins. Furthermore, their production is not influenced by seasonal or geographical factors, and their yield can be controlled and predicted [1], [2]. The investigation of microbial pigment synthesis has recently emerged as a prominent area of scientific research. Fortunately, the prevalence of microbes capable of producing various colours is extremely high. Recently, there has been a rise in interest in microorganism-derived pigments, particularly those containing polyisoprenoid compounds like β-carotene, canthaxanthin, and astaxanthin [3]. Numerous bacterial species generate β- carotene and astaxanthin (a xanthophyll), which are crucial for preserving the retinal macula's yellow colour and enabling it to function as a sunscreen on specific retinal regions [4]. Microorganisms produce a variety of colored pigments to shield their cells from the damaging effects of visible and near-ultraviolet light as well as other stressful factors like pH, temperature, etc. [5].

Kocuria has been isolated from dairy products, beer, human skin, and animals. They can also be present in

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water, dust, and soil, among other parts of the environment. Kocuria is able to survive in conditions with little water or high levels of salt [6], [7]. The isolated and recognized bacterium Kocuria produces a natural orangish-yellow coloured pigment that was extracted and used for a variety of purposes, including fabric dyeing, visual living art, candles, paper, nail paint, sketch pens, antibacterial activities, and bio-ink. Carotenoids, a class of pigments, are classified as producing colours in the yellow-orange spectrum [8], [9]. Carotenoids are classified as tetraterpenoids, consisting of 40 carbon atoms, which are composed of four terpene units, each containing 10 carbon atoms. They are polyene hydrocarbon chains that can occasionally end in rings and that, depending on their structure, may or may not be further modified with oxygen atoms. Their coloring, which ranges from pale yellow to bright orange to deep red, has a direct correlation with their structure [10], [11].

Due to their availability, microbial carotenoids are currently used as the primary source for the manufacture of pigments. Additionally, it offers several favourable biological features for humans. In biological systems, they serve as both a chemoprotective agent and a provitamin A [12]. Additionally, they exhibit antibacterial and antioxidant effects [13], [14], [15].

Some of the bacteria's pigments have antibacterial properties that are effective against pathogens. These antimicrobial agents or substances made by the bacteria are effective in both treating and preventing microbial diseases. The antibacterial effects of pigments such as carotenoids, melanin, flavin, violacein, and prodigiosin were clearly noticeable against several harmful bacteria [4], [16], [17], [18], [19]. Carotenoids are used therapeutically to treat conditions such as cancer, cataracts, and cardiovascular diseases [20], [21]. The present investigation aimed to isolate and identify pigment-producing strains, extract pigment from the isolated strain, characterize the pigment chemically, and evaluate the antioxidant, antibacterial, and antibiofilm qualities of the bacterial pigment.

2. Materials and Methods

2.1. Organism Source and Isolation

The isolated Pigment-producing strain in our study was isolated from the soil of a petroleum well in Egypt. The serial dilution and spread plate technique were used with A nutrient agar (NA) medium containing 5% glycerol was used for the isolation, which was carried out and incubated for 24-48 hours at 37°C. Bacteria exhibiting pigmentation were identified through the presence of visibly colored colonies, which diffused pigments over agar plates. The pure-colored isolates were picked and stored in a NA medium; the bacterial isolates underwent further sub-cultures. For further research, pure isolates were shifted to agar tubes and stored at 4 °C.

2.2. Molecular identification of the promising isolate

Phytogenicanalysis was used to validate the identity [22]. Macrogen Sequencing Facilities carried out the DNA sequencing of the PCR product. Subsequently, the PCR products underwent sequencing using the primers 5'GGATTAGATACCCTGGTA3' and 5'CCGTCAATTCTTTAGTTT3', and the resulting sequence data were subjected to analysis utilizing the basic local alignment search tool (BLAST) software available at http://www.ncbi.nlm.nih.gov/blast. The bacterial 16S rDNA sequences utilized for phylogenetic analysis were obtained from taxonomically defined homologues available in the GenBank database on NCBI (http://www.ncbi.nlm.nih.gov/genbank). The DDBJ/EMBL/GenBank nucleotide sequence databases encompass the 16S rRNA gene sequences of bacteria, which are identified by the accession number OM921388.1.

2.3. Bio-production and Extraction of Pigment

Inoculum preparation and pigment production were both prepared by using Nutrient Broth media (NB). A loop full of culture was added to 50 ml of NB in 250 ml flasks, and the mixture was then incubated for 24 hours at 37 °C on a rotary shaker at 100 rpm. One millilitre of the inoculum size was added to a 250 ml Erlenmeyer flask that contained 50 ml of NB+ 5% glycerol, and the mixture was then incubated at 37 °C for 72 hours on a rotating shaker at 100 rpm [23]. The culture medium was collected and subjected to centrifugation in order to separate and eliminate cell pellets. In order to extract the bacterium dye from the cell, 10 mL of methanol (with a purity of 99.7%) was added to the cell pellets then vortexed for 5 minutes after that, the pigment extract was separated by centrifugation at 5,000 rpm for 10 minutes. The pigment extract was then filtered using Whatman filter paper to create a suitable sample for measuring absorbance. The solvent evaporated to dryness at 37°C, at which point the crude dye was weighed and collected in a light-tight sealed bottle [24].

2.4. Characterization of yellow pigment

2.4.1. LC-ESI-MS/MS

Instrument: The CYP extraction has been evaluated using liquid chromatography, electrospray ionization, and tandem mass spectrometry. For ion separation, an Exion LC AC system has been employed, followed

by positive and negative ion detection using a SCIEX Triple Quad 5500+ MS/MS system using electrospray ionization (ESI). **Positive ionization mode:** The separation technique was performed using an Ascentis® Express 90 Å C18 Column (2.1 × 150 mm, 2.7 µm). Two eluents, X (ammonium format, 5 mM, pH 3) and Y (acetonitrile, LC grading), were used for developing the mobile phases. The mobile phase had been set up as follows: (5–100%) Y from 1 to 20 min, (100%) Y from 20 to 25 min, 5% at 25.01, and 5% from 25.01 to 30 min. The injection volume was 5 µl, and the flow rate was 0.3 ml/min. Positive ionization mode has been applied with an EMS-IDA-EPI scan (from 100 to 1000 Da) for MS1, with the subsequent settings for MS/MS evaluation: Ion source gases one and two were from 50 to 1000 Da and 45 psi, and for MS2, collision energy (35), declustering potential (80), collision energy spread (15), Ion Spray voltage (500); curtain gas (25psi), and source temperature (500°C). **Negative ionization mode:** The separation technique was performed using an Ascentis® Express 90 Å C18 Column (2.1 × 150 mm, 2.7 µm). Two eluents, X (ammonium format, 5 mM, pH 8) and Y (acetonitrile, LC grading), were used for developing the mobile phases. The mobile phase had been set up as follows: (5-100%) Y from 1 to 20 min, (100%) Y from 20 to 25 min, 5% at 25.01, and 5% from 25.01 to 30 min. The injection volume was 5 µl, and the flow rate was 0.3 ml/min. negative ionization mode has been applied with an EMS-IDA-EPI scan (from 100 to 1000 Da) for MS1, with the subsequent settings for MS/MS evaluation: Ion source gases one and two were from 50 to 1000 Da and 45 psi, and for MS2, collision energy (-35), declustering potential (-80), collision energy spread (15), IonSpray voltage (-4500), and curtain gas (25 psi). MS-DIAL was employed for the identification of compounds.

2.4.2. ***UV-visible spectrophotometer***

The CYP methanol solution's UV spectra were scanned in the 200–800 nm wavelength range using a JASCO V-730 UV-visible/NIR double-beam spectrophotometer (Tokyo, Japan).

2.4.3. ATR-FTIR spectroscopy

The JASCO FTIR 6100 spectrometer (Tokyo, Japan) has been employed to record the ATR-FTIR transmission measurements of the freeze-dried CYP spectrum in the range of 4000-400 cm^{-1} region with 60 scans and a resolution of 4 cm^{-1}.

2.5. Antioxidant potential of extracted pigment

A radical-scavenging activity (1-diphenyl-2-picrylhydrazyl) assay was used to assess the yellow pigment's antioxidant capability. The ability of the pigment to donate hydrogen or to scavenge free radicals using DPPH was used to determine its antioxidant properties. according to Brand-Williams *et al*.'s method [25]. CYP, at various concentrations of 2.0, 4.0, 6.0, 8.0, and 10 mg/ml, and ascorbic acid, at different concentrations (20–100 µg/ml), were prepared with methanol serving as the solvent. Further, 1 mL of 0.1 mM DPPH solution in methanol was added to 1 mL of samples and incubated in the dark for varying lengths of time (30, 60, 90, and 120 min). A 517 nm measurement was made to determine the sample's absorbance by using a UV-Vis spectrophotometer (UV-2401PC SHIMADZU) compared to the blank (methanol) and 0.1 mM DPPH in methanol as a negative control. The reaction was conducted in triplicate. Using the formula below, the inhibition percent was determined. % Of scavenging activity = Ac-As/Ac100 Where Ac represents the control's absorption and As represents the absorbance of the sample [25].

2.6. Antimicrobial activity of yellow pigment extract

To measure the CYP antimicrobial activity. Gram-positive bacteria (*Staphylococcus aureus* NRRLB-767 and *Bacillus Subtilis* ATCC 6633), Gram-negative bacteria (*Escherichia Coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 10145), yeast (*Candida albicans* ATCC 10231), and fungi (*Aspergillus niger* NRRLA-326) were used as test organisms and antimicrobial tests. On 96-well flat polystyrene plates, the tests were conducted. 10µl of yellow pigment (final concentration of 500 µg/ml) were added to 80 µl of lysogeny broth (LB broth) with the following composition (g L^{-1}): tryptone, 10.0; yeast extract, 5.0; NaCl, 10.0. At pH 7.2, followed by the addition of 10 µl of bacterial culture suspension, and then the plates were incubated overnight at 37 °C. After incubation, the positive antibacterial effect of the pigment was observed as clearance in the wells, while compounds that didn't influence the bacteria appeared opaque in the wells. The absorbance was measured after 20 h at OD 600 in a Spectrostar Nano Microplate Reader (BMG LABTECH GmbH, Allmendgrun, Germany)[26], [27].

2.7. Calculating the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentrations(MBC)

MIC is measured by preparing 5 ml of NB that can be sterilized in test tubes to confirm the MIC of a bacterial pigment. Each tube was then filled with 0.1 ml of bacterial culture and varying amounts of a pure pigment that had been cultivated for 24 h in a rotary shaker at 150 rpm and 37 °C. Microbial development was calculated at 620 nm, and the outcomes were displayed in terms of proportion, expressed as a percentage of the inhibitory zone. whereas the only component of the control sample is a bacterial

culture. By sub-culturing from plates having concentrations equal to or more significant than the MIC on fresh broth culture, the MBC was found. The tubes were incubated at a temperature of 37 °C for a duration of 18 to 24 h. The various tubes were subsequently distributed on fresh nutrient agar plates, and then the samples were subjected to incubation at a temperature of 37 °C for an additional duration of 18 to 24 h to identify noticeable agar colonies. The minimal concentration of the extract was determined, and no bacterial growth was seen**.**

2.8. In vitro antibiofilm activity

In 96 wells of flat-bottom polystyrene plates with two clinical microorganisms (*E. Coli* ATCC 25922 and *S. aureus*NRRLB-767), the pigment extract's biofilm inhibiting activity was assessed using the microtiter plate assay (MTP) technique according to [29], [30]**.**Each well was filled with 180 µL of (LB) lysogeny broth and 10 µL of overnight testing for bacterial growth, the plate was subsequently incubated for 24 h at 37 °C with 10 µL of CYP at a concentration of 100 µg mL^{-1} and the negative control (i.e., filtrate without sample). To get rid of the floating bacteria, the contents of each well were evacuated, and each well was washed with 200 µL of phosphate buffered saline, pH 7.2. The plate was stained for 1 hour with 0.1% (w/v) crystal violet applied to each well; 200 µL of distilled water was used to wash, and then it was allowed to dry in a laminar flow. 95% ethanol was applied to the dry plate, and a SPECTROSTAR nano absorbance plate reader (BMG LABTECH) was employed to quantify the optical density (OD) at 570 nm.

2.9. Statistical analysis

The mean values are shown for each experiment, which were all run in triplicate. The findings were reported as mean ± SD. With IBM SPSS Statistics 20, statistical analyses were carried out.

3. Results and Discussion

3.1. Isolation and molecular characterization of the isolate GMA

Based on the morphological differences (colour) on agar plates, a pure culture of bacteria that produce yellow pigment was isolated from the soil of a petroleum well; the GMA isolate was selected for additional research as a yellow pigment producer based on the greatest intensity of the extracted pigment in the 200–800 nm wavelength range as scanned by the spectrophotometer. Carotenoids may be the pigment created, based on the maxima of a yellow pigment's spectrum scan at 465 nm. GMA colonies were observed to have a yellow color, possess a smooth texture, and display a circular shape with slightly convex surfaces and intact edges. Furthermore, they were characterized by their gram-positive nature, aerobic metabolism, non-motile behaviour, and coccoid cellular morphology [31]. Additionally, these specimens exhibited positive catalase activity **(Fig. 1)**. The strain GMA nucleotide sequence was identified and maintained in GenBank with accession number OM921388.1. The construction of the phylogenetic tree was undertaken based on the 16S rDNA sequence using the neighbour joining technique **(Fig. 2).** A BLAST comparison was performed between the closely full 16S rRNA gene sequence of the GMA strain and the sequences of comparable bacterial strains from the GenBank database. The BLAST results showed that the GMA strain and *Kocuria sp.* were 100% similar. A phylogenetic tree was built by applying the neighbour-joining method. The *Kocuria sp.* GMA strain's membership in the Kocuria genus is strongly supported by the tree's structure and strong bootstrap values. GMA was identified as *Kocuria sp.* GMA based on the examination of the 16S rDNA sequencing data and morphological traits.

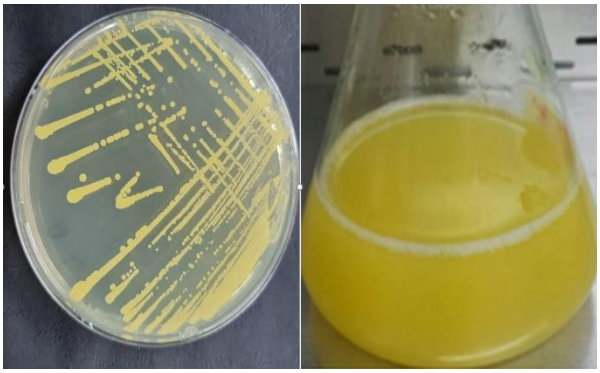

Figure 1: Yellow pigment from isolated bacteria GMA**.**

3.2. Bio-production of pigment

CYP extracted from isolated strain GMAwith methanol (99.7%) showed a yellow color in **Fig. 1.** The productivity percent of crude pigment extract was 60.13%, where the derided crude Pigment weight was 72.4 mgand the cell dry weight was 120.42 mg.

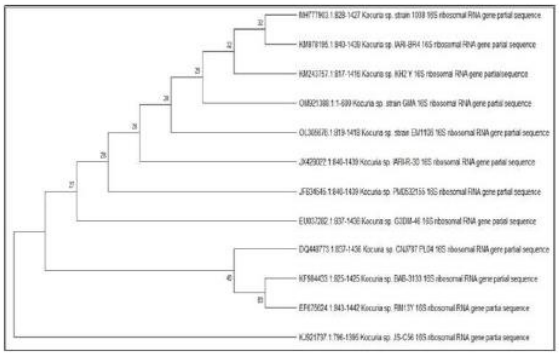

Figure2: Phylogenetic tree of *Kocuria* sp. GMA obtained by neighbor-joining analysis**.**

3.3. Characterization of yellow pigment

In the pigment extract of *Kocuria* SP. bacteria, seven compounds, mostly carotenoids, were identified. The recognition depended on the molecular weights, fragmentation, and available identified compounds in the literature. The obtained LC-MS spectra **(Fig. 3 and Table 1)** depicted the presence of molecular ion peaks at m/z [M+H] 224.04, 536.05, 553.22, 540.17, and 704.27, which lead to a molecular weight of 224, 536, 552, 538.9, and 704 g/mol, which refer to Kocumarin, Lycopene, beta-cryptoxanthin, Neurosporene, and Flavuxanthin, respectively; molecular ion peaks at m/z [M-H] lead to a molecular weight of 705.67 and 223.06, which refers to Kocumarin and Sarcinaxanthin, respectively.

Brahmaa and Dutta [32] isolated beta-cryptoxanthin (b-CRX), a provitamin A carotenoid, from *Kocuria marina* DAGII. The UV-Vis spectrometer was employed to identify the absorbance characteristic bands of the carotenoid extract between 350 and 650 nm. Also, Mendes-Silva et al. extracted carotenoids from *Kocuria palustris*. The isolated carotenoid compounds were identified using liquid chromatography (HPLC) and mass spectrometry (LC-MS)[33]. Kocumarin was isolated from Actinobacterium Kocuria marina by Uzair et al. [34]. Rezaeeyan and colleagues, isolated methnolic carotenoids with anticancer properties from *Kocuria* sp. UV/light absorption spectrophotometry and mass spectrometry are used to characterize the extracted pigments. The extracted carotenoid MTT experiment was performed on seven cancer cell lines[35].

Kocumarin is a unique benzoic acid derivative, and the absorption spectra of *Kocuria sp.* methanolic extract are depicted in **Fig. 4.** According to the spectrum, it is obvious that the extract from *Kocuria sp.* exhibits fingerprints of three distinctive peaks in the region around 400 and 500 nm, which characterize the carotenoid components. The extracted yellow pigments from *Kocuria sp.* show an extensive maximal absorption band with three distinct shoulders (peaks) in the blue spectrum at 410, 435, and 465 nm, respectively[34], [36], [37]. The carotenoid pigments are responsible for absorbing light in the visible spectrum.

The ATR-FTIR spectrum of *Kocuria sp.* yellow extract exhibited major peaks at 3290, 2928, 1621, 1450, and 1039 cm^{-1} as demonstrated by **Fig. (5).** Unfortunately, there is relatively limited research on bacterial carotenoids identified through the FTIR technique. The figure demonstrated a broad peak from 3723 to 2994 cm^{-1} that corresponds to the hydrogen-bonded OH group's starching vibration. The existence of such a particular peak might assist in clarifying how the pigment seems to be extremely soluble in methanol and ethanol [38]. The bands ranging from 2990 to 2883 cm^{-1} are caused by elongating or extending symmetrical and asymmetrical vibrations of the CH_3 group (aliphatic). Furthermore, the FTIR spectrum of *Kocuria sp.* yellow extract showed distinctive polyene characteristic bands, involving peaks at 1621 cm^{-1} corresponding to C=C stretching as well as at 1039 cm^{-1} corresponding to C-C stretching, demonstrating the presence of aliphatic units [39]. It's also possible that the peak at 1621 cm^{-1} also contributed to the carbonyl C=O stretch ketone's stretching vibration [40]. The band at 1450 cm^{-1} is caused by the scissoring vibration of methylene -CH_2 which could be observed in the beta-carotene standard at 1450.68 cm^{-1}. This might be related to the abundance of lycopene carotenoids [41]. The spectra peak emerged at 1150 cm^{-1}, attributable to the stretching vibration of ester's C-O [42].

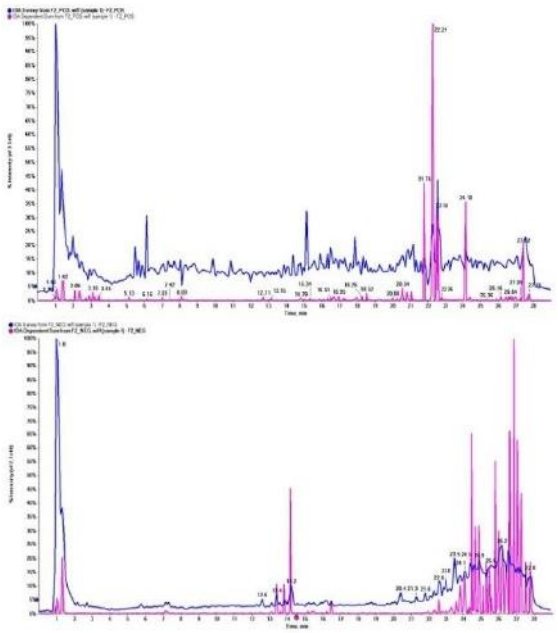

Figure 3: LC-MS profile of the methanolic extract of *Kocuria*sp

Table 1: Identification of Carotenoidsin *Kocuria* sp. GMAExtract

Peak	RT (min)	[M+H] +/ [MH]- (m/z)	MS/MS (m/z)	Tentative Identification	Reference
		M+H			
1	0.731	224.04	149,121, 108	Kocumarin	**(Uzair et al., 2018)**
2	4.528	536.05	536, 414, 280, 148	Lycopene	**(Mendes-Silva et al.,2021)**
3	6.71	553.22	553,536,139	β-Cryptoxanthin	**(Samanta et al., 2016), (Brahma et al., 2022)**
4	7.713	540.17	307, 215, 200	Neurosporene	**(Rezaeeya et al., 2017)**
5	27.152	704.27	563, 493, 285, 297	Flavuxanthin	**(Netzer et al., 2010)**
		M-H			
6	5.410	223.06	223, 207, 134	Kocumarin	**(Uzair et al., 2018)**
7	26.655	705.67	388, 241, 253, 153	Sarcinaxanthin	**(Mendes-Silva et al.,2021)**

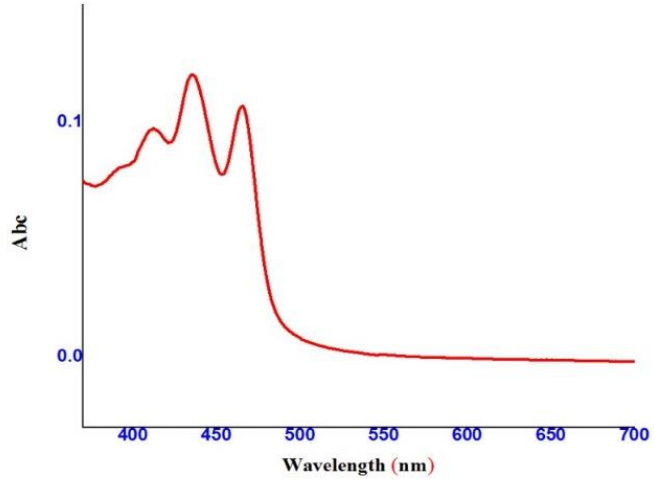

Figure 4: UV-Vis spectrum of CYP isolated from *Kocuria sp.* GMA

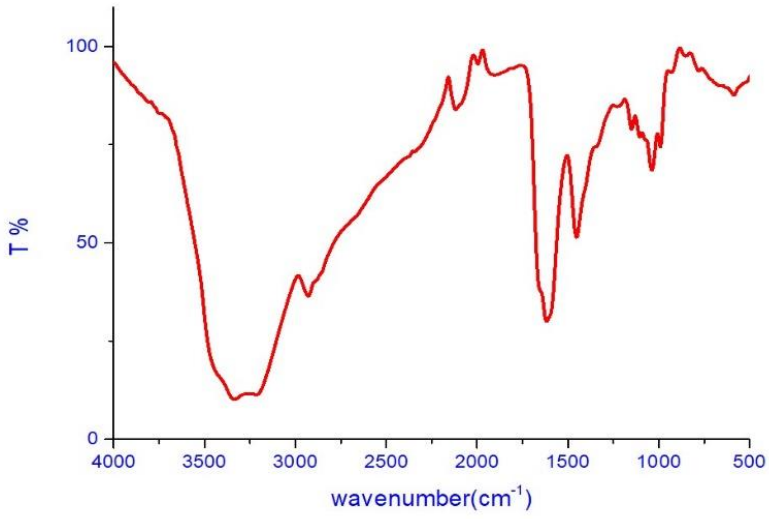

Figure 5: ATR.FTIR spectrum of CYP isolated from Kocuria sp. GMA

3.4. Antioxidant potential of CYP

The antioxidant activity was assessed by evaluating the CYP's ability to neutralize DPPH, a stable freeradical.Consequently,thepresenceofantioxidantsled todecreased absorbance values, causing decolorization. DPPH radical scavenging capacityofthe CYP methanolic extract is assessed in relation to ascorbic acid. The activity of ascorbic acid was shown to be relatively high, with an IC_{50} value seen at a concentration below 20 µg/mL after 30 minutes. The alterations in DPPH radical scavenging activity (RSA) of the extracted pigment are shown in **Fig 6,** showcasing a range of concentrations (2.0, 4.0, 6.0, 8.0, and 10.0 mg/ml) and varied incubation durations in darkness (30, 60, 90, and 120 min). The radical scavenging activity of CYP on DPPH exhibited significant activity in a manner that depends on the dosage and is influenced by the duration of incubation in the absence of light. Specifically, when CYP was present at a 10 mg/ml concentration, the radical scavenging activity reached 95.6% after 120 minutes in the absence of light. Additionally, the IC_{50} values were determined to be 4.0 mg/ml at 90 minutes and 6.0 mg/ml at 60 minutes. The total antioxidant capacity was quantified and expressed in terms of ascorbic acid equivalents [43]. The findings suggest that the pigment exhibits a comparatively lower level of antioxidant activity in comparison to normal ascorbic acid. In recent times, there has been a notable increase in the investigation of naturally derived antioxidants as substitutes for synthetic antioxidants. The latter have faced limitations owing to their potential to induce carcinogenic effects [44]. The researchers in the study conducted by Mal et al., [38] identified that the yellow pigment derived from *Kocuria flava* MN162713 exhibited DPPH radical scavenging activity. The in vitro antioxidant experiment revealed an IC_{50} value of 1.25 mg/ml for this activity. The IC_{50} value of the carotenoids generated by *K. flava*majod, as determined by Mercy and Aruna, was found to be 3.2 (mg/mL) in their antioxidant study[45]. In a separate investigation, the carotenoid pigments derived from *M. roseus* and *M. luteus* had a noteworthy capacity for UV protection and demonstrated an antioxidant IC_{50} value ranging from 3.5 to 4.5 mg/ml [46]. The carotenoid with the highest production, obtained from the *Exiguobacterium* sp. strain extracted from both soil and air sources, was chosen for its scavenging activity, which exhibited an approximate efficacy of 70% [47]. Carotenoids have significant potential as antioxidants, particularly *in vivo*. However, carotenoid molecules have been found to have a substantial role in exhibiting antioxidant and anticarcinogenic characteristics [48]. The observed disparity in IC values can be explained by the presence of acyclic carotenoids that possess a substantial quantity of conjugated double bonds and hydroxyl groups inside the primary carotenoid compound [49]. Carotenoid pigments are reported for their biological activities. Beta-cryptoxanthin showed good antioxidant properties[32] and exhibited antiproliferative activity, which is achieved through the reduction in cell viability, inhibition of cell migration, and induction of G0/G1 arrest. Sarcinaxanthin exhibits promising antioxidant and photoprotective activities [50]. Lycopene was reported to improve antioxidant parameters such as catalase andGlutathione reduced form (GSH) levels and also exhibit good anti-inflammatory activity [50], [51]. Neurosporene and lycopene have been reported for their good antiproliferative activity [52]. The results of this study are important to note and highlight. The pigment obtained from the *Kocuria sp.* GMA isolate holds potential for use in industrial applications following the process of purification.

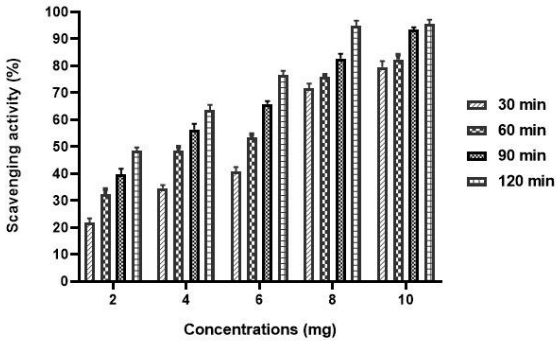

Figure 6:DPPH scavenging activity of CYP

3.5. Antimicrobial and antibiofilm activities of CYP

The continual development of current knowledge regarding the management of diseases caused by pathogens is of the utmost priority, given the increasing incidence of bacterial strains that demonstrate resistance to routinely utilized antibiotics. Furthermore, there are dynamic trends in the susceptibility of microorganisms to commercially available antimicrobial drugs [53], [54]. So, the antimicrobial activity of CYP isolated from *Kocuria* sp. GMA was assessed using the 96-well flat polystyrene plates method. Various concentrations of the pigment were employed in the experiment. The CYP that was subjected to isolation exhibited varying degrees of antibacterial activity against the tested pathogens at varied doses. The results indicated a modest level of effectiveness against gram-positive and gram-negative microorganisms. The results demonstrated a high level of antifungal efficacy against *A. niger* NRRLA-326 **(Table 2)**.

Table 2: Anti-microbial activity of CYP

compounds	Antimicrobial activity (%)					
	Gram positive		Gram negative		Yeast	Fungi
	S. aureus **NRRLB-767**	***B. Subtilis*** **ATCC 6633**	***E. Coli*** **ATCC 25922**	***P. aeruginosa*** **ATCC 10145**	***C. albicans*** **ATCC 10231**	***A. niger*** **NRRLA-326**
CYP	56.80±0.76	48.22±0.98	65.40±1.15	69.00±0.98	12.85±1.15	90.30±1.19
Ciprofloxacin	96.90 ± 0.86	91.63 ± 0.55	98.40 ± 0.22	98.87 ± 0.14	-	-
Nystatin	-	-	-	-	97.27 ± 0.34	98.27 ± 0.11

The MIC and MBC values of the pigments against bacteria that are both Gram-positive and Gram-negative exhibited a range of 10 to 50 µg/ml, as shown in **Table 3**. The lowest MIC and MBC values, specifically 10 and 40 µg/ml, were observed exclusively against gram-positive bacteria, namely*S. aureus* NRRLB-767 and *B. subtilis* ATCC 6633. Hence, the MIC and MBC exhibited significantly greater values in gram-negative bacteria, specifically *E. Coli* ATCC 25922 and *P. aeruginosa* ATCC 10145. The previously mentioned outcome was reached through the consensus of Rashid et al. [55] who successfully recovered 15 bacteria capable of colour formation from soil samples. These bacteria were subsequently identified at the genus level as *Pseudomonas, Flavobacterium, Chromobacterium, Xanthomonas, Aeromonas, Escherichia,* and *Bacillus*. All of the pigments exhibited broad-spectrum inhibitory effects against both gram-negative and gram-positive bacteria. MIC values of the pigments were observed to range between 1500 and 4000 µg/ml. while, Dawoud et al. [56] conducted a study on the synthesis of yellow pigment derived from endolichenic*Bacillus* sp., which was obtained from the lichen *Dirinariaaegialita*. Subsequently, an examination was conducted on a total of twenty distinct strains that possess the ability to produce yellow pigment. The antifungal activity was evaluated by measuring the suppression of mycelial growth in the fungus. In this study, the antifungal properties of both crude and pure pigments were assessed following a three-day incubation period against three fungal strains, namely *R. solani*, *F. oxysporum*, and *S. rolfsi*. The observed pigment extract activity varied among the various fungi. The extracted pigment exhibited significant inhibitory activity against *S. rolfsi* and *R. solani*. Mal et al. [38] reached the conclusion that *Kocuria flava* SIF3 isolate was able to create yellow pigments with broad-spectrum antibacterial action against a number of human infections.Since antibiofilm is one of the primary causes of bacterial medication resistance. Therefore, research on the antibiofilm activity against various Gram-positive and Gram-negative bacteria (*S. aureus* NRRLB-767 and *E. coli* ATCC 25922) revealed that *S. aureus* NRRLB-767 exhibited excellent activity while *E. coli* ATCC 25922 exhibited moderate activity **(Table 4)**. These findings are consistent with those of Naisi et al. [57], who contend that the pigment from *R. glutinis* is effective in eliminating both the aquatic free-floating and biofilm-forming forms of food spoilage bacteria (*Staphylococcus aureus* and *Salmonella typhimurium*). *S. aureus* and *S. typhimurium* had geometric mean MIC values of 4.1 µl.ml^{-1} and 17.9 µl.ml^{-1}, respectively. Additionally, the geometric means of MBC for *S. aureus* and *S. typhimurium* were reported to be 8.1 µl.ml^{-1} and 23 µl.ml^{-1}, respectively. While the MtP assay revealed that the pigment's 1/2MIC and 1/4MIC concentrations were crucial in preventing all bacteria from forming biofilms. Therefore, Bin et al. [58] investigated the effect of the *A. auricula* melanin MIC values for *E. coli* K-12, *P. aeruginosa* PAO1, and *P. fluorescens* P-3 and found that there was a decrease in biofilm biomass with an increase in *A. auricula* melanin concentration at a sub-MIC of 80 µg ml^{-1} inhibited biofilm formation of *E. coli* K-12, *P. aeruginosa* PAO1 and *P. fluorescens* P-3 up to 71.3, 61.7, and 63.2%, respectively.

Table 3: MIC and MBC of the CYP

compounds	MIC & MBC (µg/mL)							
	Gram positive				Gram negative			
	S. aureus **NRRLB-767**		***B. Subtilis*** **ATCC 6633**		***E. Coli*** **ATCC 25922**		***P. aeruginosa*** **ATCC 10145**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
CYP	10	40	10	40	20	40	20	50
Ciprofloxacin	5.0	-	5.0	-	3.90	-	7.50	-

Table 4: Antibiofilm activity of the CYP

	Biofilm inhibition ratio (%)	
	***E. Coli* ATCC 25922**	***S. aureus* NRRLB-767**
CYP	68.95848	96.86862

4. Conclusion

Carotenoid yellow pigment (CYP), which was isolated from Kocuria sp. GMA on the NA and glycerol 5% growth medium, had the best pigment quality and quantity.Additionally, spectrophotometry employing FT-IR, UV, and LC-ESI-MS/MS was used to corroborate the CYP characterization. In the pigment extract of *KocuriaSP*. bacteria, there are seven compounds, mostly carotenoids (Kocumarin, Lycopene, Beta-cryptoxanthin, Neurosporene, Flavuxanthin, Kocumarin, and Sarcinaxanthin). CYP exhibits antioxidant activity, as demonstrated by the DPPH assay, and this activity grew progressively at higher dosages and longer incubation times. It also possesses antimicrobial and antibiofilm activity against tested pathogens. In addition, MIC and MBC were calculated, and it was demonstrated that the CYP effectively inhibited the growth of bacteria, especially as antifungal and antibiofilm. So, the extensive uses of CYP derived from *KocuriaSP*. are evident, since its unique features have the potential to initiate advancements in various sectors of biotechnology. Our recommendation was to do additional research to enhance pigment productivity on a larger scale and reduce production costs by utilizing agro-industrial wastes. Moreover, use microbial pigment in several industrial fields.

5. Conflicts of interest:"There are no conflicts to declare"

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7. References

[1] T. Nakashima, M. Kurachi, Y. Kato, K. Yamaguchi, and T. Oda, "Characterization of bacterium isolated from the sediment at coastal area of Omura Bay in Japan and several biological activities of pigment produced by this isolate," *Microbiol Immunol*, vol. 49, no. 5, pp. 407–415, 2005, doi: 10.1111/j.1348-0421.2005.tb03744.x.

[2] Z. Qin, X. Wang, S. Gao, D. Li, and J. Zhou, "Production of Natural Pigments Using Microorganisms," *J. Agric. Food Chem.*, vol. 71, no. 24, pp. 9243–9254, Jun. 2023, doi: 10.1021/acs.jafc.3c02222.

[3] L. Dufossé *et al.*, "Microorganisms and microalgae as sources of pigments for food use: a scientific oddity or an industrial reality?," *Trends in Food Science & Technology*, vol. 16, no. 9, pp. 389–406, Sep. 2005, doi: 10.1016/j.tifs.2005.02.006.

[4] C. K. Venil and P. Lakshmanaperumalsamy, "An insightful overview on microbial pigment, prodigiosin," *eJBio*, vol. 5, no. 3, pp. 49–61, Jan. 2009.

[5] S. Sinha, S. Choubey, A. Ajay Kumar, and P. Bhosale, "Identification, Characterization of Pigment Producing Bacteria from Soil and Water and Testing of Antimicrobial Activity of Bacterial Pigments," *Int. J. Pharm. Sci. Rev. Res.*, vol. 42, no. 2, pp. 119–124, 2017.

[6] W. E. Kloos, T. G. Tornabene, and K. H. Schleifer, "Isolation and Characterization of Micrococci From Human Skin, Including Two New Species: Micrococcus lylae and Micrococcus kristinae1," *International Journal of Systematic and Evolutionary Microbiology*, vol. 24, no. 1, pp. 79–101, 1974, doi: 10.1099/00207713-24-1-79.

[7] G. Kovács, J. Burghardt, S. Pradella, P. Schumann, E. Stackebrandt, and K. Màrialigeti, "Kocuria palustris sp. nov. and Kocuria rhizophila sp. nov., isolated from the rhizoplane of the narrow-leaved cattail (Typha angustifolia)," *Int J Syst Bacteriol*, vol. 49 Pt 1, pp. 167–173, Jan. 1999, doi: 10.1099/00207713-49-1-167.

[8] P. Ratnakaran, M. Bhoir, and A. Durve, "Various applications of extracted of bacterial pigments," *World Journal of Pharmacy and Pharmaceutical Sciences*, vol. 9, pp. 1305–1323, May 2020, doi: 10.20959/wjpps20205-16086.

[9] G.-D. López, G. Álvarez-Rivera, C. Carazzone, E. Ibáñez, C. Leidy, and A. Cifuentes, "Bacterial Carotenoids: Extraction, Characterization, and Applications," *Crit Rev Anal Chem*, vol. 53, no. 6, pp. 1239–1262, 2023, doi: 10.1080/10408347.2021.2016366.

[10] A. J. Alija, N. Bresgen, O. Sommerburg, C. D. Langhans, W. Siems, and P. M. Eckl, "Cyto- and genotoxic potential of beta-carotene and cleavage products under oxidative stress," *Biofactors*, vol. 24, no. 1–4, pp. 159–163, 2005, doi: 10.1002/biof.5520240119.

[11] A. V. Rao and L. G. Rao, "Carotenoids and human health," *Pharmacol Res*, vol. 55, no. 3, pp. 207–216, Mar. 2007, doi: 10.1016/j.phrs.2007.01.012.

[12] N. Mezzomo and S. R. S. Ferreira, "Carotenoids Functionality, Sources, and Processing by Supercritical Technology: A Review," *Journal of Chemistry*, vol. 2016, p. e3164312, Mar. 2016, doi: 10.1155/2016/3164312.

[13] M. P. Narsing Rao, M. Xiao, and W.-J. Li, "Fungal and Bacterial Pigments: Secondary Metabolites with Wide Applications," *Front Microbiol*, vol. 8, p. 1113, Jun. 2017, doi: 10.3389/fmicb.2017.01113.
[14] N. Pandey, R. Jain, A. Pandey, and S. Tamta, "Optimisation and characterisation of the orange pigment produced by a cold adapted strain of Penicillium sp. (GBPI_P155) isolated from mountain ecosystem," *Mycology*, vol. 9, no. 2, pp. 81–92, Jan. 2018, doi: 10.1080/21501203.2017.1423127.
[15] G. S. Ibrahim, S. A. Abdelhamid, E. A. Elmansy, M. M. S. Asker, and F. N. El Shall, "Red pigment from isolated *Serratia marcescens* SEM: Structure, antimicrobial and antioxidant activity," *Biocatalysis and Agricultural Biotechnology*, vol. 54, p. 102932, Nov. 2023, doi: 10.1016/j.bcab.2023.102932.
[16] S. Cang, M. Sanada, O. Johdo, S. Ohta, Y. Nagamatsu, and A. Yoshimoto, "High production of prodigiosin by Serratia marcescens grown on ethanol," *Biotechnology Letters*, vol. 22, no. 22, pp. 1761–1765, 2000, doi: 10.1023/A:1005646102723.
[17] D. Kim *et al.*, "Biosynthesis of antibiotic prodiginines in the marine bacterium Hahella chejuensis KCTC 2396," *J Appl Microbiol*, vol. 102, no. 4, pp. 937–944, Apr. 2007, doi: 10.1111/j.1365-2672.2006.03172.x.
[18] A. Mortensen, "Carotenoids and other pigments as natural colorants," *Pure and Applied Chemistry*, vol. 78, no. 8, pp. 1477–1491, Jan. 2006, doi: 10.1351/pac200678081477.
[19] F. Pantanella, F. Berlutti, C. Passariello, S. Sarli, C. Morea, and S. Schippa, "Violacein and biofilm production in Janthinobacterium lividum," *Journal of Applied Microbiology*, vol. 102, no. 4, pp. 992–999, 2007, doi: 10.1111/j.1365-2672.2006.03155.x.
[20] A. S. Hermanns, X. Zhou, Q. Xu, Y. Tadmor, and L. Li, "Carotenoid Pigment Accumulation in Horticultural Plants," *Horticultural Plant Journal*, vol. 6, no. 6, pp. 343–360, Nov. 2020, doi: 10.1016/j.hpj.2020.10.002.
[21] L. Novoveská, M. E. Ross, M. S. Stanley, R. Pradelles, V. Wasiolek, and J.-F. Sassi, "Microalgal Carotenoids: A Review of Production, Current Markets, Regulations, and Future Direction," *Mar Drugs*, vol. 17, no. 11, p. 640, Nov. 2019, doi: 10.3390/md17110640.
[22] M. Gardes and T. D. Bruns, "ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts," *Molecular Ecology*, vol. 2, no. 2, pp. 113–118, 1993, doi: 10.1111/j.1365-294X.1993.tb00005.x.
[23] T. Boontosaeng, S. Nimrat, and V. Vuthiphandchai, "Pigments Production of Bacteria Isolated From Dried Seafood and Capability to Inhibit Microbial Pathogens," *IOSR-JESTFT*, vol. 10, no. 5, pp. 30–34, 2016.
[24] D. Asker and Y. Ohta, "Production of canthaxanthin by extremely halophilic bacteria," *J Biosci Bioeng*, vol. 88, no. 6, pp. 617–621, 1999, doi: 10.1016/s1389-1723(00)87089-9.
[25] W. Brand-Williams, M. E. Cuvelier, and C. Berset, "Use of a free radical method to evaluate antioxidant activity," *LWT - Food Science and Technology*, vol. 28, no. 1, pp. 25–30, Jan. 1995, doi: 10.1016/S0023-6438(95)80008-5.
[26] M. M. Abdel-Monsef, D. A. Darwish, H. A. Zidan, A. A. Hamed, and M. A. Ibrahim, "Characterization, antimicrobial and antitumor activity of superoxide dismutase extracted from Egyptian honeybee venom (Apis mellifera lamarckii)," *Journal of Genetic Engineering and Biotechnology*, vol. 21, no. 1, p. 21, Feb. 2023, doi: 10.1186/s43141-023-00470-4.
[27] H. T. Khazaal *et al.*, "Antimicrobial, antiproliferative activities and molecular docking of metabolites from Alternaria alternata," *AMB Express*, vol. 13, no. 1, p. 68, Jul. 2023, doi: 10.1186/s13568-023-01568-1.
[28] M. Asker, S. E. El-gengaihi, E. M. Hassan, M. A. Mohammed, and S. A. Abdelhamid, "Phytochemical constituents and antibacterial activity of Citrus lemon leaves," *Bulletin of the National Research Centre*, vol. 44, no. 1, p. 194, Nov. 2020, doi: 10.1186/s42269-020-00446-1.
[29] S. A. AboElmaaty *et al.*, "Biofilm Inhibitory Activity of Actinomycete-Synthesized AgNPs with Low Cytotoxic Effect: Experimental and In Silico Study," *Microorganisms*, vol. 11, no. 1, p. 102, Dec. 2022, doi: 10.3390/microorganisms11010102.
[30] M. E. Elawady, A. A. Hamed, W. M. Alsallami, E. Z. Gabr, M. O. Abdel-Monem, and M. G. Hassan, "Bioactive Metabolite from Endophytic Aspergillus versicolor SB5 with Anti-Acetylcholinesterase, Anti-Inflammatory and Antioxidant Activities: In Vitro and In Silico Studies," *Microorganisms*, vol. 11, no. 4, p. 1062, Apr. 2023, doi: 10.3390/microorganisms11041062.
[31] J. Vora, N. Jain, and H. Modi, "Identification and characterization of pigment producing strain Kocuria KM243757 & JO1 KM216829 from Kharaghoda soil," Sep. 2015.
[32] D. Brahma and D. Dutta, "Antioxidant property of beta-cryptoxanthin produced by Kocuria marina DAGII," *Materials Today: Proceedings*, vol. 57, no. 4, pp. 1833–1837, Jan. 2022, doi: 10.1016/j.matpr.2022.01.027.
[33] T. de C. D. Mendes-Silva *et al.*, "Production of carotenoid sarcinaxanthin by Kocuria palustris isolated from Northeastern Brazil Caatinga soil and their antioxidant and photoprotective activities,"

Electronic Journal of Biotechnology, vol. 53, pp. 44–53, Sep. 2021, doi: 10.1016/j.ejbt.2021.05.004.

[34] B. Uzair *et al.*, "Isolation, purification, structural elucidation and antimicrobial activities of kocumarin, a novel antibiotic isolated from actinobacterium Kocuria marina CMG S2 associated with the brown seaweed Pelvetia canaliculata," *Microbiol Res*, vol. 206, pp. 186–197, Jan. 2018, doi: 10.1016/j.micres.2017.10.007.

[35] Z. Rezaeeyan, A. Safarpour, M. A. Amoozegar, H. Babavalian, H. Tebyanian, and F. Shakeri, "High carotenoid production by a halotolerant bacterium, Kocuria sp. strain QWT-12 and anticancer activity of its carotenoid," *EXCLI J*, vol. 16, pp. 840–851, Jun. 2017, doi: 10.17179/excli2017-218.

[36] E. L. Ashenafi, M. C. Nyman, J. T. Shelley, and N. S. Mattson, "Spectral properties and stability of selected carotenoid and chlorophyll compounds in different solvent systems," *Food Chemistry Advances*, vol. 2, p. 100178, Oct. 2023, doi: 10.1016/j.focha.2022.100178.

[37] R. Netzer, M. H. Stafsnes, T. Andreassen, A. Goksøyr, P. Bruheim, and T. Brautaset, "Biosynthetic pathway for γ-cyclic sarcinaxanthin in Micrococcus luteus: heterologous expression and evidence for diverse and multiple catalytic functions of C(50) carotenoid cyclases," *J Bacteriol*, vol. 192, no. 21, pp. 5688–5699, Nov. 2010, doi: 10.1128/JB.00724-10.

[38] S. A. Mal, G. S. Ibrahim, M. I. A. Khalaf, A. M. Al-Hejin, N. M. Bataweel, and M. Abu-Zaid, "Production and Partial Characterization of Yellow Pigment Produced by Kocuria flava Isolate and Testing its Antioxidant and Antimicrobial activity.: Life Sciences-Biochemistry for better diagnosis and therapy," *International Journal of Life Science and Pharma Research*, vol. 10, no. 2, pp. 58–66, 2020, doi: 10.22376/ijpbs/lpr.2020.10.2.L58-66.

[39] J. J. Alvares and I. J. Furtado, "Characterization of multicomponent antioxidants from Haloferax alexandrinus GUSF-1 (KF796625)," *3 Biotech*, vol. 11, no. 2, p. 58, Feb. 2021, doi: 10.1007/s13205-020-02584-9.

[40] B. V. Latha, K. Jeevaratnam, H. S. Murali, and K. S. Manja, "Influence of growth factors on carotenoid pigmentation of Rhodotorula glutinis DFR-PDY from natural source," *IJBT*, vol. 4, pp. 353–357, Jul. 2005.

[41] N. Trivedi, S. Tandon, and A. Dubey, "Fourier transform infrared spectroscopy (FTIR) profiling of red pigment produced by Bacillus subtilis PD5," *AJB*, vol. 16, no. 27, pp. 1507–1512, Jul. 2017, doi: 10.5897/AJB2017.15959.

[42] M. R. A. Manimala and R. Murugesan, "Characterization of carotenoid pigment production from yeast Sporobolomyces sp. and their application in food products," *J Pharmacogn Phytochem*, vol. 7, no. 1S, pp. 2818–2821, 2018.

[43] K. P. Renukadevi and M. Vineeth, "antimicrobial, antioxidant and cytotoxicity activity of pigment produced from serratia marcescens," *European Journal of Biomedical and Pharmaceutical Sciences*, vol. 4, no. 2, pp. 265–269, Jan. 2017.

[44] Y. F. Sasaki *et al.*, "The comet assay with 8 mouse organs: results with 39 currently used food additives," *Mutat Res*, vol. 519, no. 1–2, pp. 103–119, Aug. 2002, doi: 10.1016/s1383-5718(02)00128-6.

[45] J. Mercy and K. Aruna, "Optimization of pigment production by Kocuria flava majod isolated from garden soil.," *Journal of Global Biosciences*, vol. 8, no. 2, pp. 5946–5965, 2019.

[46] D. C. Mohana, S. Thippeswamy, and R. U. Abhishek, "Antioxidant, antibacterial, and ultraviolet-protective properties of carotenoids isolated from Micrococcus spp.," *Radiation Protection and Environment*, vol. 36, no. 4, p. 168, Oct. 2013, doi: 10.4103/0972-0464.142394.

[47] P. Sasidharan, R. Raja, C. Karthik, S. Ranandkumar, and A. Indra, "Isolation and characterization of yellow pigment producing Exiguobacterium sps.," *J Biochem Tech*, vol. 4, no. 4, pp. 632–635, 2013.

[48] A. Bendich, "Carotenoids and the immune response," *J Nutr*, vol. 119, no. 1, pp. 112–115, Jan. 1989, doi: 10.1093/jn/119.1.112.

[49] F. Mandelli, V. S. Miranda, E. Rodrigues, and A. Z. Mercadante, "Identification of carotenoids with high antioxidant capacity produced by extremophile microorganisms," *World J Microbiol Biotechnol*, vol. 28, no. 4, pp. 1781–1790, Apr. 2012, doi: 10.1007/s11274-011-0993-y.

[50] M. Gao, F. Dang, and C. Deng, "β-Cryptoxanthin induced anti-proliferation and apoptosis by G0/G1 arrest and AMPK signal inactivation in gastric cancer," *Eur J Pharmacol*, vol. 859, p. 172528, Sep. 2019, doi: 10.1016/j.ejphar.2019.172528.

[51] N. M. Alhoshani *et al.*, "Antioxidant and anti-inflammatory activities of lycopene against 5-fluorouracil-induced cytotoxicity in Caco2 cells," *Saudi Pharmaceutical Journal*, vol. 30, no. 11, pp. 1665–1671, Nov. 2022, doi: 10.1016/j.jsps.2022.09.011.

[52] L. H. Campestrini, P. S. Melo, L. E. P. Peres, R. C. Calhelha, I. C. F. R. Ferreira, and S. M. Alencar, "A new variety of purple tomato as a rich source of bioactive carotenoids and its potential health benefits," *Heliyon*, vol. 5, no. 11, p. e02831, Nov. 2019, doi: 10.1016/j.heliyon.2019.e02831.

[53] G. C. Brandão, E. G. Kroon, M. G. R. Duarte, F. C. Braga, J. D. de Souza Filho, and A. B. de Oliveira, "Antimicrobial, antiviral and cytotoxic activity of extracts and constituents from Polygonum

spectabile Mart," *Phytomedicine*, vol. 17, no. 12, pp. 926–929, Oct. 2010, doi: 10.1016/j.phymed.2010.03.004.
[54] S. Gibbons, "Plants as a Source of Bacterial Resistance Modulators and Anti-Infective Agents," *Phytochem Rev*, vol. 4, no. 1, pp. 63–78, Jan. 2005, doi: 10.1007/s11101-005-2494-9.
[55] M. M. Rashid, M. Fakruddin, R. M. Mazumdar, F. Kaniz, and M. A. Chowdhury, "Anti-Bacterial Activity of Pigments Isolated From Pigment-Forming Soil Bacteria," *Journal of Pharmaceutical Research International*, vol. 4, no. 8, pp. 880–894, Feb. 2014, doi: 10.9734/BJPR/2014/5148.
[56] T. M. Dawoud *et al.*, "Characterization and antifungal activity of the yellow pigment produced by a Bacillus sp. DBS4 isolated from the lichen Dirinaria agealita," *Saudi J Biol Sci*, vol. 27, no. 5, pp. 1403–1411, May 2020, doi: 10.1016/j.sjbs.2019.11.031.
[57] S. Naisi, M. Bayat, T. Zahraei Salehi, B. Rahimian Zarif, and R. Yahyaraeyat, "Antimicrobial and anti-biofilm effects of carotenoid pigment extracted from Rhodotorula glutinis strain on food-borne bacteria," *Iran J Microbiol*, vol. 15, no. 1, pp. 79–88, Feb. 2023, doi: 10.18502/ijm.v15i1.11922.
[58] L. Bin, L. Wei, C. Xiaohong, J. Mei, and D. Mingsheng, "In vitro antibiofilm activity of the melanin from Auricularia auricula, an edible jelly mushroom," *Ann Microbiol*, vol. 62, no. 4, Art. no. 4, Dec. 2012, doi: 10.1007/s13213-011-0406-3.