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 *Kocuria* sp. 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, Neuproseorene, 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: *Kocuria* sp., 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...
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 antifouling 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

Phylogenetic analysis 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’GGATTAGATAACCCTGGTA3’ and 5’CCGTCAATTCTTATAGT TT3’, 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 x 150 mm, 2.7 µm). Two eluents, X (ammonium format, 5 mM, pH 3) and Y (acetoniitrate, 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 x 150 mm, 2.7 µm). Two eluents, X (ammonium format, 5 mM, pH 8) and Y (acetoniitrate, 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 property 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. aureusNRRLB-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 GMA with 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 mg and the cell dry weight was 120.42 mg.
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 carotenoids 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 stretching 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 CH₂ 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 -CH₂ 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].
Table 1: Identification of Carotenoids in *Kocuria* sp. GMA Extract

<table>
<thead>
<tr>
<th>Peak</th>
<th>RT (min)</th>
<th>[M+H]+/ [MH]- (m/z)</th>
<th>MS/MS (m/z)</th>
<th>Tentative Identification</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.731</td>
<td>224.04</td>
<td>149,121, 108</td>
<td>Kocumarin</td>
<td>(Uzair et al., 2018)</td>
</tr>
<tr>
<td>2</td>
<td>4.528</td>
<td>556.05</td>
<td>536, 414, 280, 148</td>
<td>Lycopene</td>
<td>(Mendes-Silva et al., 2021)</td>
</tr>
<tr>
<td>3</td>
<td>6.71</td>
<td>553.22</td>
<td>553, 536, 139</td>
<td>β-Cryptoxanthin</td>
<td>(Sumanta et al., 2016), (Brahma et al., 2022)</td>
</tr>
<tr>
<td>4</td>
<td>7.713</td>
<td>540.17</td>
<td>307, 215, 200</td>
<td>Neurosporene</td>
<td>(Rezaeeva et al., 2017)</td>
</tr>
<tr>
<td>5</td>
<td>27.152</td>
<td>704.27</td>
<td>563, 493, 285, 297</td>
<td>Flavuxanthin</td>
<td>(Netzer et al., 2010)</td>
</tr>
<tr>
<td>6</td>
<td>5.410</td>
<td>223.06</td>
<td>223, 207, 134</td>
<td>Kocumarin</td>
<td>(Uzair et al., 2018)</td>
</tr>
<tr>
<td>7</td>
<td>26.655</td>
<td>705.67</td>
<td>388, 241, 253, 153</td>
<td>Sarcinaxanthin</td>
<td>(Mendes-Silva et al., 2021)</td>
</tr>
</tbody>
</table>

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 free radical. Consequently, the presence of antioxidants resulted in a decreased absorbance value, causing decolorization. DPPH radical scavenging capacity of the CYP methanolic extract was assessed in relation to ascorbic acid. The activity of ascorbic acid was shown to be relatively high, with an IC₅₀ 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 IC₅₀ 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 IC₅₀ value of 1.25 mg/ml for this activity. The IC₅₀ value of the carotenoids generated by K. flavamajod, 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 IC₅₀ 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 within their molecular structure. 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 and glutathione 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).
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 NRRL-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 Dirinariaeagialita. 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 flavia SIF3 isolate was able to create yellow pigments with broad-spectrum antibacterial action against a number of human infections. Since antibiotic 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 NRRL-767 and E. coli ATCC 25922) revealed that S. aureus NRRL-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>
<thead>
<tr>
<th>compounds</th>
<th>Antimicrobial activity (%)</th>
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<tbody>
<tr>
<td>S. aureus NRRL-767</td>
<td>B. Subtilis ATCC 6633</td>
</tr>
<tr>
<td>MIC</td>
<td>MBC</td>
</tr>
<tr>
<td>CYP</td>
<td>10</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5.0</td>
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</table>

Table 2: Anti-microbial activity of CYP

Table 3: MIC and MBC of the CYP

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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 biofilm 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 antibiotic. 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


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