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Biological activities and chemical characterization of orange carotenoids pigment from psychotropic *Planomicrobium sp.* GMMA isolate

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Abstract

Carotenoids of natural origin are classified as secondary metabolites and are known to exhibit features such as anti-inflammatory, anti-cancer, and antioxidant effects. The exploration of novel natural sources of carotenoids has been driven by the demand for these compounds in several industries, including food, textiles, cosmetics, and pharmaceutical. This research is intended to study the isolation and identification of strains producing orange carotenoid pigment (OCP), the extraction of the pigment from the isolated strain, the chemical characterization of the pigment and testing its antioxidant, antimicrobial and antibiofilm properties. By using morphological traits and 16S rRNA sequencing, the isolate was recognized as *Planomicro-bium sp.* GMMA, with accession number OM921394.1. OCP from *Planomicrobium sp.* GMMA was first confirmed for purity and characterization by UV, FTIR and LC-ESI-MS/MS spectrophotometer. In the pigment extract of *Planomicrobium sp.*, nine compounds were tentatively identified, including eight carotenoid derivatives and one chlorophyll. The obtained LC-MS spectra showed molecular ion peaks at m/z [M+H] 536.05, 224.1, 553.22, 570.1, 499.5, 600.16, 644.2 and 706.2, which lead to a molecular weight of 536, 224, 552, 568.9, 497.7, 600, 642.9, and 704 g/mol, which refers to Lycopene, Kocumarin, β-Cryptoxanthin, zeaxanthin, Neurosporaxanthin, Violaxanthin, Dinoxanthin and Flavuxanthin, respectively. The molecular ion peaks at m/z [M-H] 895.2, which leads to a molecular weight of 895.47 g/mol, which refers to chlorophyll. OCP had considerable radical scavenging activity with DPPH, ABTS, ferrous ions (Fe^{2+}), and nitric oxide. Also, OCP showed good antimicrobial activity against the microorganism's test. The MIC and MBC values of OCP against Gram-positive and Gram-negative bacteria ranged from 10 to 40 µg/ml. Therefore, the antibiofilm activity of the OCP was moderate; it showed 58.41 and 54.73% for *S. aureus* and *E. coli*, respectively.

Keywords: *Planomicrobium sp.*, orange carotenoid pigment, chemical characterization, biological activities

1. Introduction

Both synthetic and natural pigments are widely used in a wide range of industries, such as food, feed, paper, textiles, printing ink, cosmetics, and pharmaceuticals. Pigments play a vital role as colour additives within the food business, as the perception of colour significantly influences customer acceptance of food products. Consequently, a multitude of synthetic food dyes have been developed, a significant proportion of which are associated with deleterious health consequences. Concerns about the toxicity of some artificial colourants have led to a rise in the usage of natural additives in recent years. Consumer awareness of natural colourants obtained from fruits, vegetables, roots, and microorganisms has increased, resulting in a greater emphasis on their manufacture [1]. The production of colours by microbes is a relatively new phenomenon. The term "microbial pigments" descry-bes the process of producing chromatic compounds using microbial organisms. Bacterial pigments are well acknowledged for their enhanced environmental compatibility and superior biodegradability [2]. There exist two categories of colourants that fall under the umbrella of carotenoid pigments: natural and synthetic. Artificial colorings pose a significant threat to both the environment and human health due to their poisonous nature and inability to undergo natural decomposition. The need for natural colours derived from plants, animals, fungus, and microbes is experiencing a notable increase[3]. Research has demonstrated that bacterial pigments include antibacterial, antioxidant, and anticancer properties, hence suggesting their potential significance as additions for food colouring purposes [4–6]. Within the spectrum of colours reportedly generated by bacteria, there exist two

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distinct cate-gories of naturally occurring pigments, namely caro-tenoids and xanthophylls. These pigme-nts are known to exhibit distinct hues of yellow and orange, respectively [7]. Several microorganisms have been identified as carotenoid producers, including *Micrococcus, Paracoccus, Flavobacterium*, *Bacillus*, *Spor-obolomyces*, *Rhodotorula*, *Paffia* and *Blakeslea trispora* [5]. Carotenoids repre-sent a prevalent class of naturally occurring pigments that can be observed in several plant species and microorganisms [7]. Carotenoids have widespread application across several industries due to their inherent content of vitamin A and antioxidants, as well as their charact-eristic yellow, orange, and red hues. This pigment comprises around 700 unique chemical compounds exhibiting atypical coloration and possessing distinctive biological characteristics [8]. Carotenoids, a class of lipophilic isoprenoid molecules, possess conjoined double bonds that merge to generate light-absorbing chromophores, so conferring them with their characteristic colouring attributes [9]. Carotenoids are prone to isomerization, oxidation, photo degradation, thermal degradation, acid-catalyzed reactions, and oxidative reactions as a result of the presence of many double bonds [10]. Carotenoids have been observed to potentially facilitate the modulation of bacterial membrane fluidity, enabling their survival in cold conditions. This is achieved by the absorption or obstruction of UV light, so serving as a protective mechanism for bacterial cells [11]. *Planococcus* bacteria are aerobic, Gram-positive, motile cocci that are members of the *Micrococcae* family. These bacteria are widespread in a variety of settings, frequently in severe ones like the psychrosphere. Certain strains of the *Planococcus* species have been identified as the producers of the uncommon C30 carotenoids. On the other hand, the production mechanism of C30 carotenoids is still mostly unknown. However, these carotenoids have significant antioxidative activity and are intriguing variables influencing stem cell growth, which makes these metabolites more appealing in the biotech nology field [12], [13]. Additionally, *Planoco-ccus* spp. was employed to create carotenoids from waste materials, such as cellulose pulp, which could reduce the cost of producing pigments [14]. The aims of the present investigation encompassed isolating and identifying psychotropic strains producing orange pigment, extracting and analysis of pigment properties and evaluation of its antioxidant capacity, antimicrobial and antibiofilm properties.

2. Materials and Methods

2.1. Organism's source and isolation

The strain of pigment-producing microorganisms investigated in our study was obtained from the ambient air within our laboratory, located in Egypt. The isolation procedure involved the use of nutrient agar (NA) medium supplemented with 5% glycerol. The samples were cultured at room temperature under cold weather conditions for duration of 24-48 h. The identification of pigment-producing bacteria was achieved through the observation of coloured colonies, which were diffusible on agar plates. The coloured isolates with high purity were carefully selected and afterwards preserved in a nutrient agar (NA) medium.

2.2. Identification of selected isolate

The isolate chosen for examination was identified using Bergey's Guide to Specific Bacteriology. The isolated colonies with an orange pigment (GMMA) were taken to analyze their morphological, biochemical, physiological, and 16S rRNA sequencing characteristics. The genomic DNA was extracted from the bacterial isolate and subsequently assessed for quality using a 1.2% agarose gel. Upon examination, a solitary band of high-molecular-weight DNA was detected. The rese-archers conducted a polymerase chain reaction (PCR) using the primers 5'GG ATTAGATACCCT GGTA3' and 5'CCGTCAATTC TTTAGTTT3' [15]. Upon resolution on an agarose gel, a solitary and distinct polymerase chain reaction (PCR) amplicon band was detected. The PCR amplicon underwent a purification process in order to eliminate any potential impurities. The PCR amplicon underwent forward and reverse DNA sequencing reactions, followed by 35 amplification cycles. Each cycle consisted of denaturation at 94°C for 45 seconds, annealing at 55°C for 60 seconds, and extension at 72°C for 60 sec. In the case of bacteria, DNA fragments undergo amplification of approx.-imately 1,400 base pairs. It is recommended to incorporate a positive control, which includes E. coli genomic DNA, and a negative control, in the PCR experimental design. The sequencing process was conducted utilizing the Big Dye terminator cycle sequencing kit, manufactured by Applied Biosystems in the United States. The sequencing products were resolved using an Applied Biosystems model 3730XL automated DNA sequencing device (Applied Biosystems, USA) and then sent to Macrogen, South Korea, for sequencing. The data were submitted to the GenBank database. The DNA sequence was subjected to a comparative analysis using the GenBank database, which is housed in the National Centre for Biotechnology Information (https://www.ncbi.nlm.nih.gov/). This analysis was conducted using the BLAST program [16]. The alignment of selected sequences exhibiting the highest degree of resemblance to the 16S rRNA sequencing of the bacterium strain was performed, resulting in the generation of a phylogenetic tree. The bacterial 16S rRNA gene sequences were uploaded to the DDBJ/-EMBL/GenBank nucleotide sequence databases under the accession number OM921394.1.

2.2. Bio-production and extraction of pigment

The utilization of nutrient broth (NB) medium has been employed for the purposes of inoculum preparation and pigment generation. A culture containing Loop was introduced into 50 ml of NB solution within 250 ml Erlenmeyer flasks. Subsequently, the flasks were placed on a rotary shaker operating at 100 rpm and incubated at 20 °C for 24 h. A volume of 1 ml of inoculum was put into a 250-ml Erlenmeyer flask containing 50 ml of NB supplemented with 5% glycerol. The flask was then incubated at a temperature of 20°C on a rotary shaker operating at 100 rpm for a duration of 72 h [6]. The medium underwent centrifugation at a speed of 4000 rpm for duration of 10 min in order to facilitate the separation of the cellular pellet. Subsequently, the liquid portion above the sediment, known as the supernatant, was discarded. The cell pellets were re-suspended in distilled water and then subjected to centrifugation at a speed of 4000 rpm for 10 min in order to recover the cells by removing the supernatant once again. The technique of abrasion using glass pearls was derived from the method suggested by Medeiros et al. [17]. Biomass (0.5 g) was mixed with 6 mL of acetone then frozen for 48 h after glass pearls were added and crushed in mortar, then agitated vigorously for 5 minutes by a vortex agitator. This process was repeated three times. Following cell rupture, the supernatant was isolated using centrifugation (4000 rpm for 15 min) for subsequent carotenoid extraction. After that, methanol was added to the disrupted cells and agitated vigorously for 5 minutes by a vortex agitator. This process was repeated three times to complete the extraction. The solvent was subjected to evaporation until complete dryness using an evaporator maintained at a temperature of 37°C. The resulting crude pigment was accurately weighed and subsequently transferred into a screw-cap bottle, which was then stored in a light-protected environment at a temperature of -20°C [18].

2.3. Characterization of orange pigment

The material was analyzed using liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). An Exion LC AC system was used for separation, and a SCIEX Triple Quad 5500+ MS/MS system with electrospray ionization (ESI) was used for detection. The UV spectra of the extract in a methanol solution were scanned using a JASCO V-730 UV-visible/NIR double-beam spectrophotometer (Tokyo, Japan) in the wavelength range of 200–800 nm. Visible light absorbance measurements were conducted in triplicate. The ATR-FTIR spectroscopy was condu-cted using the JASCO FTIR 6100 spectrometer from Tokyo, Japan has been employed to record the freeze-dried extract sample spectrum in the 4000-400 cm^{-1} region with 60 scans and a resolution of 4 cm^{-1}.

2.4. Antioxidant potential of extracted pigment

2.4.1. DPPH

The radical-scavenging activity of the purified pigment was assessed using the 1-diphenyl-2-picrylhydrazyl (DPPH) assay to determine its antioxidant potential. The antioxidant property of the OCP with concentrations (2.0, 4.0, 6.0, 8.0, and 10 mg/ml) were determined by assessing its potential to donate hydrogen or scavenge radicals using the DPPH technique within 30, 60, 90, and 120 min. compared with Ascorbic Acid at concentrations ranging from 20 to 100 µg/ml [19]. The measurement of the sample's absorbance was conducted at 517 nm using a UV-Vis spectrophotometer (UV-2401PC Shimadzu). The calculation of the inhibition percentage was performed using the following formula.

$$\text{Scavenging activity (\%)} = A_c\text{-}A_s/A_c\times 100$$

Where A_c is the absorbance of the control and (A_s) is the absorbance of the sample.

2.4.2. ABTS Radical Cation Scavenging Activity

The ABTS radical cation scavenging activity of various concentrations of samples (2.0, 4.0, 6.0, 8.0, and 10.0 mg/mL) was assessed and contrasted with Ascorbic Acid at concentrations ranging from 20 to 100 µg/ml.

2.4.3. Ferrous Ions (Fe^{2+}) Chelating Capacity

The sample's ability to bind Fe^{2+} ions was ascertained using the methodology established by [20]. The chelating action was observed by comparing it to a reference molecule (ascorbic acid) at concentrations ranging from 20 to 100 µg/ml.

2.4.4. Nitric Oxide Radical Scavenging Activity

The examined material's capacity to remove NO• radicals was carried out employing sodium nitroprusside (SNP) and subsequently contrasted with ascorbic acid (20-100 µg/ml) [21].

2.5. Antimicrobial activity of pigment extract

To evaluate the effectiveness of antibacterial agents of OCP, the test organisms used included bacteria that are gram-positive, specifically *Staphylococcus aureus* NRRLB-767, and *Bacillus subtilis* ATCC 6633. Two types of Gram-negative bacteria, including *Pseudomonas aeruginosa* ATCC 10145 and *Escherichia coli* ATCC 25922 were also included. Additionally, the yeast species *Candida albicans* ATCC 10231 and the fungus species *Aspergillus niger* NRRLA-326 were utilized. Antimicrobial tests were conducted following established protocols. The experiments were performed using 96-well flat polystyrene plates. A volume of 10µl of OCP, with a final concentration of 500µg/ml, was introduced into 80µl of lysogeny broth (LB broth). Subsequently, 10 µl of bacterial culture suspension in the logarithmic growth phase was added. The resulting mixture was then incubated at a temperature of 37°C for an overnight period. Following incubi-tion, the presence of the pigment was shown to have a good antibacterial impact, as evidenced by the

observed clearance in the wells. Conversely, compounds that did not affect the bacteria resulted in an opaque appearance of the growth medium in the wells. The control group consisted of the pathogen without any treatment. The measurement of absorbance was conducted around 20 h following the initiation of the experiment using a Spectrostar Nano Microplate Reader manufactured by BMG LABTE CH GmbH, located in Allmendgrun, Germany [22-24].

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

The determination of MIC involved the preparation of 5 ml of nutrient broth (NB) in sterilized test tubes. This was done in order to ascertain the MIC of OCP. Subsequently, 0.1 ml of bacterial culture and different quantities of pigment extract were added to the tubes, which had undergone 24 h cultivation using a rotary shaker at 150 rpm and 37°C, were introduced into each tube. The measureement of microbial growth was conducted at a wavelength of 620 nm, and the outcomes were expressed as a proportion of the inhibitory zone. In contrast, the control sample solely consisted of a bacterial culture. The MBC was determined by subculturing plates with concentrations equal to or greater than the MIC on a freshly prepared broth culture. The tubes were incubated at a temperature of 37°C for duration of 18 to 24 h. The different tubes were afterward allocated onto fresh nutrient agar plates and incubated at a temperature of 37°C for an additional period of 18 to 24 h in order to discern discernible colonies on the agar. The MIC of the pigment extract was established, and no bacterial growth was observed .

2.7. In vitro anti-biofilm activity

The microtiter plate assay (MTP) method was used to test the pigment extract's ability to stop biofilm formation. This involved measuring the activity on 96 well-flat bottom polystyrene titer plates and testing against two clinical microorganisms, namely *E. coli* and *S. aureus* [26]. A total volume of 180 µL of lysogeny broth (LB) was added to each well of the 96 well-plates. The LB had the following composition (g l^{-1}): tryptone, 10; yeast extract, 5.0; NaCl, 10. At a pH of 7.2, a volume of 10 µL of test bacteria that had been growing overnight, together with 10 µL of a pigment extract at a concentration of 100 µg ml^{-1}, were added to the sample. A negative control, consisting of filtrate without the sample, was also included. The plate was thereafter placed in an incubator set at a temperature of 37°C for duration of 24 hours. The contents of every well were extracted, followed by a thorough washing of each well using 200 µL of phosphate buffered saline at a pH of 7.2 in order to eradicate any microorganisms present in the liquid. A solution of crystal violet with a concentration of 0.1% (w/v) was introduced into each well for 1 hour to facilitate the staining process. Following this, 200 µL of distilled water was employed for the purpose of washing. Subsequently, the plate was placed in a laminar-flow environment to allow for drying. To determine the optical density at a wavelength of 570 nm, a solution of 95% ethanol was introduced to the desiccated plate. The optical density was then measured using a SPECTRO star nano absorbance plate reader manufactured by BMG LABTECH.

2.8. Statistical analysis

The trials were performed in triplicate, and the average values were reported. The findings were reported regarding the mean value ± SD. The statistical analyses were conducted using IBM SPSS Statistics 20.

3. Results and Discussion

3.1. Isolation and identification of the producing bacterial isolate

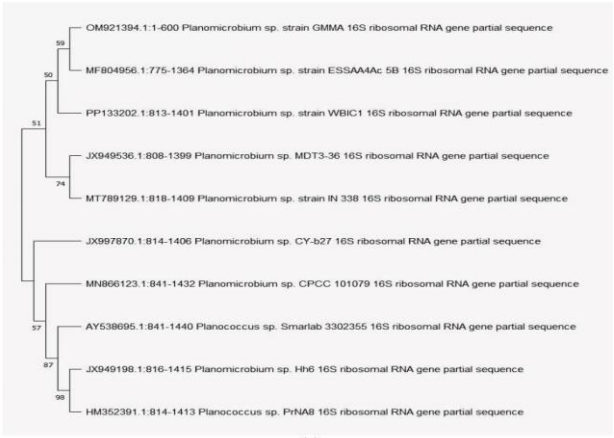
Bacterial pigmentation was prevalent and observed in several biological environments, such as air, soil, rhizospheric soil, desert sand, freshwater, and aquatic samples [27, 28]. The composition of air includes microorganisms such as bacteria, fungi, mycotoxins, and viruses. These microorganisms aggregate and persist in the atmosphere under conditions of elevated humidity [29]. The bacterial isolate consisting of a significant quantity of microorganisms that produce orange coloring was selected (Figure 1a). The GMMA isolate was selected for further examination as a potential producer of orange pigments based on the measurement of the isolated pigment's peak intensity within the spectral range of wavelengths 200–800 nm using a spectrophotometer. The spectral analysis of an orange pigment exhibited a peak at 649.2 nm, indicating the potential presence of carotenoids in the pigment (Figure 1b). Bacterial colony growth in agar media is commonly assessed and delineated based on attributes such as form and shape, elevation, margin, appearance, pigmentation (colour), and texture [30]. So, the bacterial isolate was initially identified using Bergey's Manual of Determinative Bacteriology showed that the GMMA isolate is Gram-staining-positive, aerobic, cocci or short rods, motile and non-endospore-forming. Colonies are smooth, circular, and convex, with entire margins of orange colour, while the 16S rRNA gene sequence of the MGGA isolate was subjected to a comparative analysis with sequences of bacterial strains exhibiting similarities in the GenBank database using the BLAST algorithm. The BLAST analysis revealed a complete similarity of 100% with *Planomicrobium* sp., so GMMA was identified as *Planomicrobium* sp. strain GMMA and subsequently the data has been submitted to GenBank and can be found with the entry number OM921394. The

neighbour joining method and the 16S rDNA sequence were used to generate the phylogenetic tree (Figure 1C).

3.2. Bio-production of orange carotenoid pigment

Pigment extracted from the isolated strain GMMA with methanol showed an orange colour (Figure 1b). The productivity percent of crude pigment extract was 60.5%, where the derided crude pigment weight was 69.53 mg/l and the cell dry weight was 114.95 mg/l.

(a) (b)



(c)

Figure 1. a) GMMA isolate**; b)** Orange pigment from isolated bacteria GMMA and **C)** Phylogenetic tree of *Planomicrobium* sp. strain GMMA

3.3. Identification of Planomicrobium SP. GMMA orange pigment

In the extract of *Planomicrobium* SP. GMMA, nine compounds were tentatively identified, including eight carotenoid derivatives and one chlorophyll. The recognition depended on the molecular weights, fragmentation, and available identified compounds in the literature. The obtained LC-MS spectra showed molecular ion peaks at m/z [M+H] 536.05, 224.1, 553.22, 570.1, 499.5, 600.16, 644.2 and 706.2, which lead to a molecular weight of 536, 224, 552, 568.9, 497.7, 600, 642.9, and 704 g/mol (Figure 2), which refers to Lycopene, Kocumarin, β-Cryptoxanthin, zeaxanthin, Neuro-sporaxanthin, Violaxanthin, Dinoxanthin and Flav-uxanthin, respectively (Table 1).

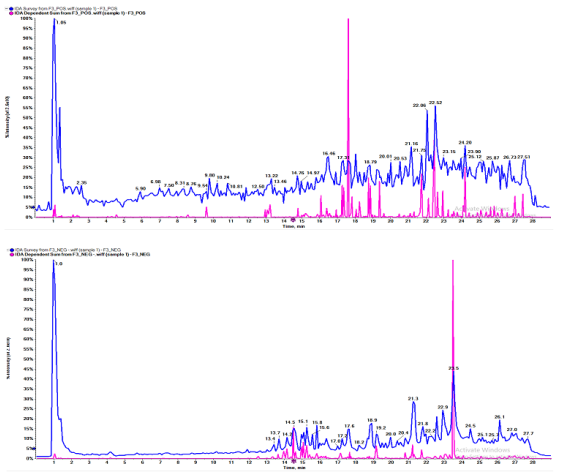


Figure 2. LC-MS profile of the methanolic extract of *Planomicrobium* SP. strain GMM

The molecular ion peaks at m/z [M-H] 895.2, which leads to a molecular weight of 895.47 g/mol, which refers to chlorophyll d. Carotenoids are the most diverse and widely distributed pigments in nature, as they exist in prokaryotic and eukaryotic organisms. Most carote-noids have a potential role in protecting the cell from photo-oxidative damage [31]. The potential of lycopene to enhance antioxidant indices, including catalase and GSH levels, has been documented. This augmentation enables cells to better manage the oxidative stress induced by 5-FU. Additionally, it demonstrated anti-inflammatory properties [32]. β-cryptoxanthin, a pro-vitamin A xanthophyll, showed good antioxidant properties [33] and exhibits antiproliferative activity by reducing cell viability, migration, and induction of G0/G1 arrest [34]. Furthermore, zeaxanthin has the ability to demon-strate its antioxidant characteristics through the direct suppression of reactive oxygen species (ROS) and the facilitation of glutathione (GSH) synthesis, thus providing protection against oxidative stress [35]. Moreover, neurosporene and lycopene revealed good antiproliferative activity in red cherries [36]. In parallel, violaxanthin exhibited remarkable lipid peroxidation-inhibitory activity and singlet oxygen-quenching activity [37], and dinoxanthin showed a protective effect against ROS [38]. On a diverse side, kocumarin is one of the notable benzoic acid derivatives that showed antibiotic properties [39]. Carotenoids play a key role in the process of photosynthesis, helping to capture light energy and transfer it to chlorophyll [40]. Chlorophyll dye has been reported to have two distinct absorption bands in the UV spectrum, which absorb visible light and lie in both the blue region between 410 and 480 nm (soret band) and the red region between 660 and 680 nm (Q band) of the spectrum. Furthermore, the carotenoids have a recognizable band with three shoulders that may be observed between 400 and 500 nm [41, 42].

The UV-visible spectrum of the crude methanol-extracted pigment solution produced by *Planomicrobium* SP is depicted in Figure (3a). The curd extract's absorption spectra exhibit two absorption bands: one in the 620–670 nm regions (the absorption maximum appeared at 649.2 nm) and a broad absorption band between 420–480 nm. A broader band that can be observed in the blue spectrum around 420 and 480 nm may result from the overlap between the carotenoids' Three Shoulders band and the band of chlorophyll [43]. The verification of functional groups of phytocompounds in the *Planomicrobium* SP extract was recognized through the infrared spectral technique. Figure (3b) depicts the ATR-FTIR spectrum of a freeze-dried *Planomicrobium* SP extract. A major peak that ranges from 3065 to 3562 cm^{-1} can be utilized for identifying both O-H and N-H stretching, which could assist in clarifying why pigment is so readily soluble in methanol and ethanol [44, 45]. The band emerging at 2923 cm^{-1} monitors symmetric and asymmetric stretching vibrations of C-H (aliphatic alkane) groups. Also, the bands at 2854 and 1376 cm^{-1} corresponded to –CH (CH_2) asymmetric stretching and bending vibrations of lycopene. There is evidence of COO functioning at the band at 1740 cm^{-1}. The bands between 1619 cm^{-1} and 1418 cm^{-1}, which match conventional protein patterns, are indicative of the amide II bands and show the presence of C-N and C-C vibrations as well as N-H in-plane (bending) and stretching, respectively (according to similar studies, the existence of chlorophyll is demonstrated by the FTIR bands at the same region of the spectrum) [45, 46]. Both the C-H bending from aliphatic amine and the C-O stretching from hydroxyl, flavones, esters, catechins, and amide II dominate the area between 1286 and 1207 cm1. The peak of the signal at 1043 cm1 was associated with the CO-C bond vibration, which is often observed in carbohydrates [47].

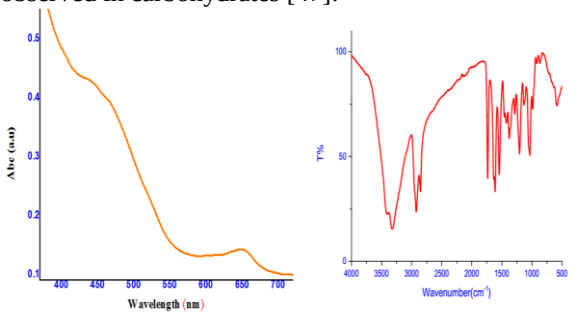


Figure 3. UV (a) and ATR-FTIR spectrum (b) of orange pigment from of *Planomicrobium* SP. GMMA.

3.4. In vitro antioxidant activity of pigment extract

The ability of the pigment methanolic extract to remove DPPH radicals is tested in comparison to ascorbic acid. The activity of ascorbic acid was shown to be relatively high, with an IC_{50} value seen at a concentration < 20 µg/mL after 30 min. Pigment extract from *Planomicrobium* sp. GMMA (OCP) had considerable DPPH scavenging activity (45.8±1.3 to 86.4±1.6% at 2–10 mg after 120 min, correspondingly) and had IC_{50} of 8.0, 7.0, 5.0 and 3.0 mg/ml at 30, 60, 90 and 120 min, respectively (Figure 4), whereas the IC_{50} of ascorbic acid was 13.17 µg/mL Supplementary Table 1. The effectiveeness of OCP in scave-nging the ABTS discoloration technique was used to assess ABTS radicals at different concentrations Table 2. The OCP shown notable action at a dose of 2 mg/mL, with a scavenging percentage of 46.81±1.12%. This percentage steadily climbed to 88.30±1.16% when the concentration was elevated to 10 mg/mL.

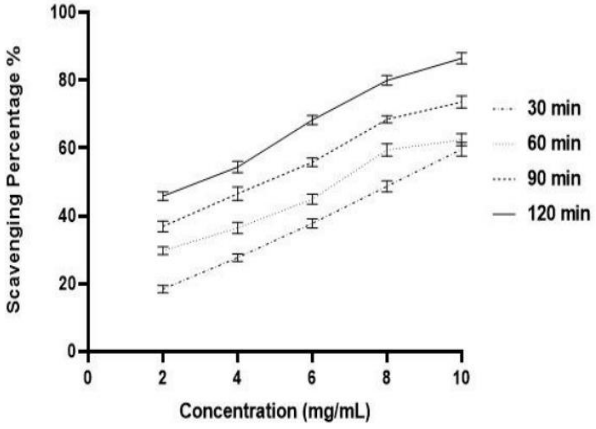


Figure 4. DPPH scavenging activity of OCP

By comparison, vitamin C exhibited scavenging percent-ages of 88.93 ± 1.25% and 99.97 ± 0.01% for the concentrations of 20 and 100 µg/mL, significantly ($p < 0.05$) in their respective order Supplementary Table 1. In the ABTS method, ascorbic acid showed an 8.65 µg/mL IC_{50}, while OCP showed an IC_{50} of 2.19 mg/ml. While, the findings of the analysis of the OCP as well as vitamin C chelation effectiveness on ferrous ions (Fe^{2+}) belong to the group of transition metals by the formation of ferrozine complexes is presented in Table 2. The findings indicated that OCP exhibited a moderate capacity to chelate ferrous ions in comparison to ascorbic acid. OCP exhibited a chelation percentage of 44.23 ± 1.17% at the lowest concentration, which climbed to 72.61 ± 1.39% at the maximum concentration. In comparison, vitamin C demonstrated chelation percentages of 83.26 ± 1.04%

and 99.28 ± 0.52% at concentrations of 20 and 100 µg/mL, respectively Supplementary Table 1. The IC_{50} values for OCP and ascorbic acid were 2.27 mg/mL and 6.32 µg/mL, respectively. Therefore, the ability of OCP to neutralize NO radicals the capacity of OCP to scavenge NO radicals was evaluated using a Single Nucleotide Polymorphism (SNP) that generates a Nitric Oxide (NO) system. According to the data in Table 2, it was determined that OCP exhibited a lower capacity to scavenge NO compared to the reference material. Nevertheless, the capability of OCP to scavenge NO was contingent upon concentration, and as the concentration of OCP gradually rose, its capacity to scavenge nitric oxide (NO) also notably elevated. At a dosage level of 2 mg/mL, OCP effectively removed 42.56 ± 1.03 % of the NO, and at the highest possible concentration of 10 mg/mL, it removed 73.49 ± 1.11 % of the NO. The values for both of these substances were lower compared to ascorbic acid, which demonstrated the ability to eliminate 89.39 ± 1.16% and 99.08 ± 0.25% of the NO at the concentrations of 20 and 100 µg/mL, respectively Supplementary Table 1.

Table 1. Identification of Carotenoids and organic compounds isolated from OCP

Peak	RT (min)	(m/z)	M+H/M-H	Fragments	Tentative Identification	Ref
1	0.809	536.05	M+H	536, 414, 280, 148	Lycopene	[57]
2	0.895	224.1	M+H	149,121, 108	Kocumarin	[39]
3	5.447	553.22	M+H	553,536	β-Cryptoxanthin	[56]
4	10.477	570.1	M+H	570, 526,467	zeaxanthin	[58]
5	16.492	499.5	M+H	499, 481, 238	Neurosporaxanthin	[59]
6	20.314	600.16	M+H	600, 446	Violaxanthin	[60]
7	21.00	644.2	M+H	644, 626, 474	Dinoxanthin	[61]
8	23.45	706.2	M+H	493,297	Flavuxanthin	[8]
9	25.07	895.2	M-H	639, 621, 607, 379	Chlorophyll d	[59]

Table 2. Antioxidant activity of OCP

Conc. (mg/mL)	ABTS radical scavenging activity	Fe^{2+} ion chelation ability activity	NO scavenging capacity
2	46.81 ±1.12	44.23 ± 1.17	42.56 ± 1.03
4	58.74 ±1.03	52.71 ± 1.28	49.41 ± 1.66
6	69.19 ±1.31	58.38 ± 1.18	57.15 ± 1.82
8	80.40 ±0.93	64.19 ± 1.07	65.38 ± 1.10
10	88.30 ±1.16	72.61 ± 1.39	73.49 ± 1.11

Data are reported as the average of three triplicates ± SD. The data analysis used a one-way ANOVA with ($n = 3$, $p < 0.05$).

Table 3. antimicrobial activity of OCP

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
OCP	93.53 ± 0.84	85.64 ± 0.99	50.74 ± 1.12	70.57 ± 0.86	12.97 ± 0.98	65.57 ± 0.88
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 concentration of OCP needed to obtain 50% of the produced NO was 2.58 mg/mL. Regarding ascorbic acid, its concentration was measured to be 8.01 µg/mL. The total antioxidant capacity was quantified and expressed in terms of ascorbic acid equivalents [48]. The findings suggest that the pigment exhibits a comparatively lower level of antioxidant activity in comparison to normal ascorbic acid. Previous studies have demonstrated the capacity of bacterial pigment to effectively scavenge DPPH radicals [49]. Carotenoids are acknowledged as antioxidants because of their possession of triplet-state energy and their electron-rich polyene structure [50]. Carotenoids possess the ability to eliminate reactive oxygen species (ROS) by means of electron transfer, oxidation, or the creation of carotenoid-radical cations. Additionally, they can impede the production of radicals by deactivating electronically stimulated sensitizer molecules [51,52].

3.5. Antimicrobial and antibiofilm activity of orange pigment extract

The antibacterial activity of the OCP was subsequently analyzed Incubation with a 500 mg/mL quantity of pigment was able to block mainly the examined disease-causing microorganisms, and plaques of the percentage of inhibition were witnessed (Table 3). The percentage inhibition findings for pathogens of both Gram-positive and Gram-negative nature were *B. subtilis* 85.64±0.99%, *S. aureus* 93.53 ±0.84%, *E. coli* 50.74±1.12% and *P. aeruginosa* 70.57±0.86%. But the antifungal activity against tested fungi was 12.97± 0.85% for *C. albicans* and 65.57±0.88 for *A. niger*. Therefore, the antibiofilm activity of the OCP was assessed against various Gram-positive and Gram-negative bacteria (*S. aureus* NRRLB-767 and *E. coli* ATCC 25922). The results revealed that *S. aureus* NRRLB-767 and *E. coli* ATCC 25922 exhibited moderate activity; they showed 58.40708 and 54.73111% for *S. aureus* NRRLB-767 and *E. coli* ATCC 25922, respectively. Pigment produced by selected bacteria at present, according to the study, showed good antibacterial activity against *B. subtilis* and *S. aureus*. A study with a comparable orange pigment was reported, wherein the treatment of penetrating bacterial infections resulted in the disruption of the cytoplasmic membrane [53]. A latest studies has found that *Planococcus maritimus* exhibits a protective effect on *Caenorhabditis elegans*, mitigating the harm resulted from *Vibrio anguillarum* [54]. The current investigation demonstrates notable efficacy in inhibiting fungal growth. Similar effects of antibacterial and antifungal properties can be observed in *A. niger*, *P. digitatum*, *A. flavus*, and *P. commune*. The impact of *Planococcus* sp., a type of halophilic bacteria, on the production of diverse secondary metabolites with antioxidant properties [55]. The MIC and MBC values of the pigments against Gram-positive and Gram-negative bacteria ranged from 10 to 50 µg/ml (Table 4). The lowest MIC and MBC values of 10 and 40 µg/ml were found against *B. Subtilis*. Therefore, the MIC and MBC values were much higher in *S. aureus*, *E. coli*, and *P. aeruginosa*. All the pigments showed a broad spectrum in terms of inhibitory activity against gram-negative and gram-positive bacteria. The MIC value of the pigments ranged from 1500 to 4000 µg/mL [56].

Table 4. MIC and MBC of OCP

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
OCP	10 / 50	10 / 40	20 / 50	20 / 50
Ciprofloxacin	5.0	5.0	3.90	7.50

4. Conclusion

The current work concluded that the isolated *Planomicrobium* sp. GMMA has the ability to produce orang carotenoid pigment. Therefore, the existence of eight carotenoid derivatives and one chlorophyll was confirmed by UV, FTIR, and LC-ESI-MS/MS spectrophotometry, which provided varied characterizations. *Planomicrobium* sp. Produces a carotenoid pigment that demonstrates strong antibacterial and antioxidant properties. This study proposes that the orange-colored bacteria *Planomicrobium* sp. could be utilized as highly effective antibacterial and antioxidant agents.

5. References

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