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Characterization of the Red Biochromes Produced by the Endophytic Fungus *Monascus purpureus* CPEF02 with Antimicrobial and Antioxidant Activities

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Abstract: Food acceptability and appeal are significantly influenced by colour. Harmful effects associated with synthetic colorants are well established, and research is currently focused on developing natural, synthetic chemical-free substitutes from fungal sources, with broad applications in food, medicine, textiles and agriculture. Additionally, the market's dearth of natural red colour substitutes requires the creation of novel red pigment alternatives from secure and scalable sources. The goal of the current research was to establish new endophytic marine fungi that are naturally occurring bio-sources of the red pigment. Based on its profuse extracellular red pigment-producing capacity, the fungus CPEF02 was selected and identified as *Monascus purpureus* CPEF02 via internal transcribed spacer (ITS) sequences and phylogenetic analysis. The chemical moieties of the pigmented extracts were identified by liquid chromatography-high resolution mass spectrometry (LC-HRMS). The optimal culture conditions for maximum pigment production were investigated by surveying various media compositions. The methanolic fungal colourant extract was shown to have substantial antibacterial and antifungal activities against anthropogenic pathogens, *Staphylococcus aureus* (MTCC 1430), methicillin-resistant *Staphylococcus aureus* (ATCCBAA811), *Salmonella typhimurium* (MTCC 3241) and *Vibrio cholerae* (N16961) at a 100 µg/mL concentration and at a 1 mg/mL concentration for *Alternaria solani* (ITCC 4632) and *Rhizoctonia solani* (AG1-IA). This extract also exhibited antioxidant activity against the 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical with an IC_{50} of 14.42 µg/mL and a Trolox equivalent antioxidant capacity of 0.571 µM Trolox/µg of the methanolic colourant extract. The findings suggested that *M. purpureus*'s pigment could be a source of an industrially useful natural red colourant.

Keywords: food colourant; *Monascus*; polyketide; bio-pigment; antimicrobial; antioxidant

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1. Introduction

Increased awareness of the potential negative environmental and health effects of synthetically produced pigments has encouraged research towards natural alternatives for the food, textile, cosmetic, pharmaceutical and other sectors. Pigments can enhance the acceptability of and increase the demand for certain products, particularly food. Commonly used synthetic food colourants, for example, allura red, carmoisine, ponceau 4R, quinoline yellow WS, sunset yellow and tartrazine, come with label warnings in some countries such as "may have an adverse effect on activity and attention in children", which discourages their use [1].

Amongst all the natural bioresources available for natural pigments, microbes have advantages over other sources due to their high stability, better yield, water solubility,

year-round availability and cost-effective scalability. Endophytic fungi, which live inside plant tissues without causing harm to them, have been found to be prolific producers of a myriad of bio-pigments with therapeutic benefits [2]. Fungal pigments are secondary metabolites that are chemically diverse in nature, including polyketides, azophilones, quinones, melanin, carotenoids, xanthophylls, flavins, monascins, violacein and indigo [3]. These metabolites are produced under stress conditions as a defence response and have a range of bioactivities including antimicrobial, cytotoxicity, anti-inflammatory, anticancer, antioxidant and anti-atherosclerotic activities.

Monascus are filamentous ascomycetes, well studied for their red and yellow bioactive pigments with a wide range of applicability in nutraceuticals, pharmaceutics and textile industries. A total of 65 pigmented compounds have been isolated from *Monascus.* These pigmented bioactive secondary metabolites are primarily azaphilonic or polyketide in chemical nature [4]. The absorption maxima studied at distinct wavelengths have identified major hues produced by this genus, for example, yellow colourants at 330–450 nm, red colourants at 490–530 nm and orange colourants at 460–480 nm [5,6]. *Monascus purpureus* is a commercially important strains and is used in food and beverages for preservation, pigment, flavour, antimicrobial, anticancer, antioxidant, anti-obesity, human health promotion and immune-enhancing properties [7]. Its pigments are thermally stable and can be cultivated via submerged or solid-state fermentations [8]. Red yeast rice is a well-known east-Asian food that has been grown by fermenting *Monascus purpureus* on rice and has cholesterol-lowering and antimicrobial properties [9].

In the present study, the endophytic fungus *Monascus purpureus* isolated from *Avicennia marina* roots from Alibaug coastal area, Maharashtra, India [10], was optimized in different growth media and extracted with different solvents for studying maximum extracellular red pigment production. The antibacterial and antifungal activities of the pigments were tested against human pathogenic bacteria and phytopathogenic fungi. Chemical characterization was carried out to estimate the compounds responsible for the colour, bioactivity and antioxidant potential of the colourant. The colourant with the highest quantity of extracellular red pigment was tested for antioxidant activity. The methanol extracted colourant exhibited biological activity against the tested systems.

2. Materials and Methods

2.1. Isolation, Cultivation and Identification of Pigment-Producing Endophytic Fungi

The strain *M. purpureus* CPEF02 (NCBI accession number MT013404.1) had been isolated earlier in the laboratory from the roots of *A. marina*. Using a light (EVOS XL Core digital inverted microscope, Life Technologies, Thermo Fisher Scientific, Maharashtra, India) and scanning electron microscopy (Carl Zeiss, Jena, Thuringia, Germany), the strain was identified morphologically and molecularly by following the protocols of Mishra and group [10]. The culture was kept on potato dextrose agar (PDA, HiMedia, Maharashtra, India) plates at 4 °C, and it was sub-cultured every 25 days. Activated fungal agar discs from seven-day-old culture plates were used for further inoculations.

2.2. Screening of Culture Media for Pigment Production and Extraction Optimization of CPEF02

In order to maximize extracellular pigment production by the CPEF02 strain, different commercially viable media compositions were studied; 500 mL flasks containing 100 mL of five different media broths were prepared. There were Czapek's extract dox Broth (CDB), malt extract broth (MEB), potato dextrose broth (PDB), Sabouraud dextrose broth (SDB) and yeast extract peptone dextrose broth (YEPD). All the chemicals were procured from HiMedia (HiMedia, Maharashtra, India). Inoculation was carried out by adding four 6 mm, 8–10-day-old PDA fungal discs, and the flasks were maintained at 28 °C for 14 days in the dark under static conditions. After about two weeks of incubation, the different medium culture broths and the mycelia were separated using a Büchner funnel and Whatman filter paper. The separated culture filtrates were dried and lyophilized for extracellular

biochrome estimation. These lyophilized powders were further used for studying the effect of medium composition on bio-hue production and spectrophotometric analysis.

2.3. Quantification of Extracellular Polyketide Red Pigment

The culture filtrates obtained from different medium broths were UV-vis spectrophotometrically analysed using an established method [10]. Briefly, the culture filtrates were diluted in deionized water and the absorption profile was examined in the wavelength range of 200–800 nm. Carmine (Sigma Aldrich India Pvt. Ltd., Karnataka, India) was used as a standard red pigment because of its importance as a natural food colorant and stable nature [11]. The pigment production in different media was compared through the quantification of polyketide-based pigment metabolites, measured at λ_{max} of Carmine red 494 nm and expressed as milligram equivalents of Carmine per litre of the medium. The extracellular pigment yield was also measured in the form of absorbance unit at 490 nm/mL (AU_{490}/mL) as 490 nm is λ_{max} of red pigments. Additionally, to determine the best solvent for the extraction of a maximum bio-pigment, 200 mL of PDB in a 1000 mL flask was inoculated with 8–10-day-old PDA fungal discs and incubated at 28 °C for 14 days in the dark under static conditions. After incubation, the media culture broths and the mycelia were separated as before using a Buchner funnel and Whatman filter paper. The separated culture filtrates were dried and lypophilized for extracellular biochrome estimates. The culture filtrate was passed through a Dianion HP20 column and eluted with methanol (MSM). Pigment extraction was performed using ethyl acetate as a solvent (MSE). The solvent extracts were rotary concentrated and lyophilized for further analysis.

2.4. Antimicrobial Assays

To investigate the antimicrobial activity of the extracted biochrome anthropogenic bacterial cultures, *Staphylococcus aureus* (MTCC737), methicillin-resistant *Staphylococcus aureus* (ATCCBAA811), *Salmonella typhimurium* (MTCC734) and *Vibrio cholerae* (N16961) were obtained from the Microbial Type Culture Collection (MTCC), Institute for Microbial Technology (IMTECH), Chandigarh, India, and maintained by sub-culturing on Muller Hinton agar (MHA) plates. Plant pathogenic fungal cultures *Alternaria solani* (ITCC4632), *Colletotrichum capsici* (ITCC6071), *Fusarium oxysporum* (ITCC6709) and *Rhizoctonia solani* (AG1-IA) were obtained from the Indian Type Culture Collection (ITCC), Indian Council of Agricultural Research–Indian Agricultural Research Institute (ICAR-IARI), New Delhi, India, and maintained on potato dextrose agar (PDA) plates.

2.4.1. Antibacterial Assay

The antibacterial activity of the culture filtrates of the strain CPEF02 was tested using a modified disc diffusion assay [12]. Primary inoculation was performed aseptically in nutrient broth (NB, HiMedia, Maharashtra, India) with 0.1% inoculum and incubated overnight at 37 °C at 180 rpm. The overnight grown cultures were used to establish the secondary cultures, to obtain logistically grown cultures at the time of the assay. Using sterile L rods, 100 µL of the secondary cultures were spread over MHA plates and five sterile 6 mm discs of Whatman no. 1 paper were aseptically placed on them; 10% dimethyl sulfoxide (Sigma Aldrich India Pvt. Ltd., Karnataka, India) was treated as a negative control, stock solutions of the samples with concentrations of 1 mg/mL (for ethyl acetate, CFDP and methanolic extracts) and 8 mg/100 µL, 4 mg/100 µL and 2 mg/100 µL (for methanolic extract) in 10% dimethyl sulfoxide were used as test samples, and 10,000 ppm stocks of antibiotics (vancomycin and chloramphenicol) were used as positive controls. Vancomycin (Sigma Aldrich India Pvt. Ltd., Karnataka, India) was used against the Gram-positive bacteria *S. aureus* and methicillin-resistant *S. aureus*, and chloramphenicol (Sigma Aldrich India Pvt. Ltd., Karnataka, India) was used against the Gram-negative bacteria *S. typhimurium* and *V. cholerae.* Five microlitres of each control and sample were loaded onto the discs, and the plates were incubated at 37 °C. After 24 h, the inhibition zones were measured. The assay was performed in triplicate to obtain statistically significant results.

2.4.2. Antifungal Assays

A dual culture assay was used as previously described [13] to determine the antifungal activity of CPEF02. Briefly, 3 mm discs of fungal cultures of CPEF02 were aseptically placed on one corner and discs of pathogenic strains were placed on the opposite side. The negative controls were plates containing only pathogenic strains. The plates were incubated for seven days at 28 °C, and the variations between the negative control and test plates were noted. Inhibition capacity was calculated by estimating the percentage decrease in the test plates (R_e) from the control plates (R_c) using $[(R_c - R_e)/R_c \times 100]$. To check the antifungal efficacy of the solvent extracts, 1 mg/mL concentrations were prepared in sterile deionized water and added to PDA media. PDA plates without any test samples were used as negative control. The test pathogens were inoculated into 3 mm discs and incubated for 7 days at 28 °C. Inhibition capacity was calculated using the same method as described above.

2.5. Antioxidant Assay

An antioxidant capacity assay was carried out as per the previously published method [10]. Briefly, a 7 mM ABTS (Sigma Aldrich India Pvt. Ltd., Karnataka, India) aqueous solution and a 2.45 mM potassium persulfate solution were combined and allowed to sit overnight at ambient temperature (25 °C) in the dark to produce ABTS+ radical cations with an initial absorbance of 0.70 ± 0.02 at 734 nm. In a final volume of 1 mL with the ABTS+ working standard, the gradient sample concentrations and standards of abscisic acid, tert-butyl hydroquinone (TBHQ), and Trolox (Sigma Aldrich India Pvt. Ltd., Karnataka, India) were used to assess antioxidant capacity. The absorbance at 734 nm was measured immediately after a 6 min incubation time at room temperature. Plotting the scavenging ability versus concentration yielded the sample concentration to scavenge 50% ABTS radicals (IC_{50}). The findings were expressed as the µM Trolox/g pigment Trolox equivalent antioxidant capacity (TEAC).

2.6. LC-HRMS Analysis

LC-MS/MS analysis was performed using an AB SCIEX TripleTOF®5600 system equipped with an Exion LC system (AB SCIEX, Framingham, MA, USA). The chromatographic separation was carried out on a reverse phase phenomenex Kinetex C_{18} (100 mm × 2.1 mm, 1.7 µm) column. Methanol with 0.1% formic acid and water (*v*/*v*) were used as the mobile phases and are referred here as eluents A and B, respectively. The multistep gradient elution program used for the chromatographic analysis was as follows: 0–35 min, 5–95% B; 35–36 min, 95–5%, which was kept constant for 40 min. The flow rate and sample injection volume were kept constant at 0.5 µL/min and 10 µL for all the samples with a fixed column temperature of 40 °C. The mass spectrometric source parameters were set as nebulizer (GS1), drying (GS2) and curtain (CUR) gases at 40, 40 and 25 psi; desolvation temperature 500 °C; and ion spray voltage 5500 (ESI+), while the compound-specific parameters, i.e., declustering potential (DP) and collision energy (CE), were kept at 100 V and 10 V, respectively. The generated data were analysed using Analyst 1.8, MultiQuant 2.0.

3. Results

3.1. Identification and Characterization of Pigment-Producing Endophytic Fungus

Endophytic isolate CPEF02, propagated on PDA plate, was morphologically identified using light and scanning electron microscopy. The fungal hyphae displayed apical growth, with white dense and velvet mycelia initially. After 7–10 days, the agar medium started to display orange to reddish colouration owing to the secretion of pigmented metabolites extracellularly in the nutrient medium (Figure 1A,B). In visible light micrographs (Figure 1C), exogenous oval, pyriform conidia with septate hyphae were observed, whereas scanning electron micrographs (Figure 1D,E) displayed spherical ascospores originating from the conidia. Our macroscopic as well as microscopic findings were in agreement with earlier studies on *M. purpureus* FTCC 5391 [14,15]. Additionally, molecular characterization studies

previously carried out [10] in our laboratory confirmed the endophytic strain CPEF02 as *Monascus purpureus* (GenBank accession number MT013404.1).

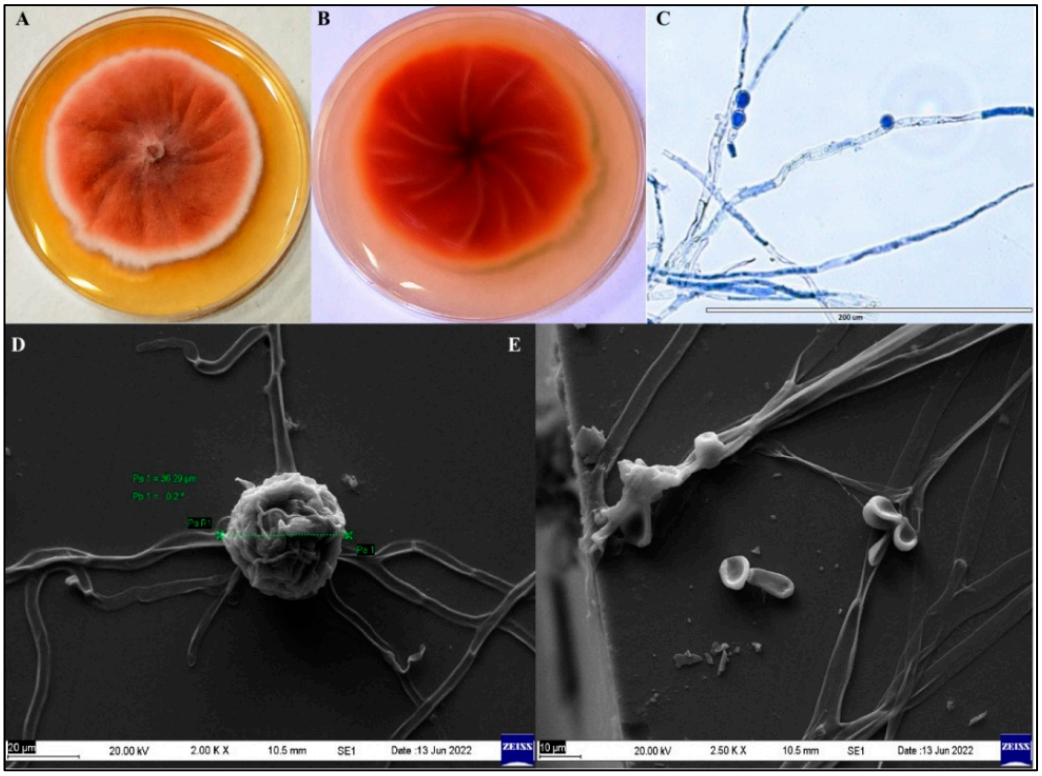
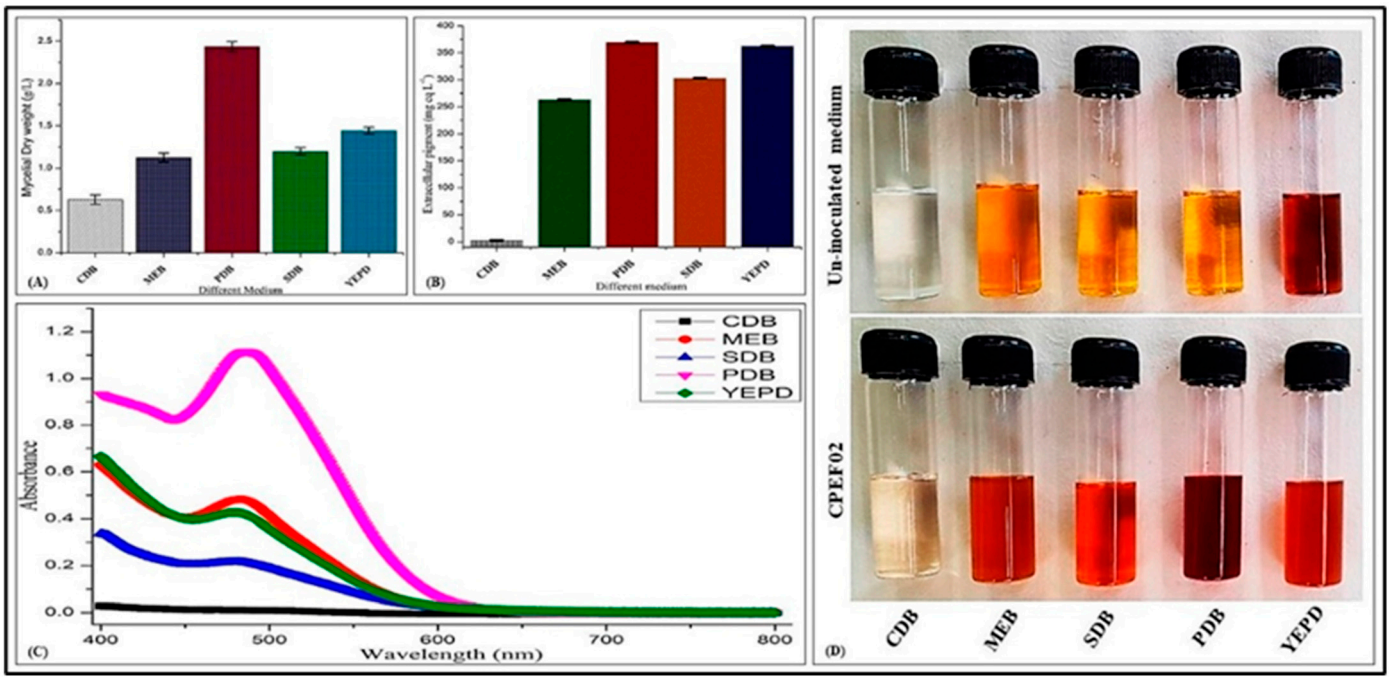


Figure 1. (**A**,**B**) Morphological images of CPEF02 after 10 days of growth at 28 °C; (**C**) light microscopic image of CPEF02 hyphae and conidia; (**D**,**E**) SEM images of CPEF02 ascospores, conidia and hyphae.

3.2. Screening of Culture Media for Pigment Production and Solvent Extraction by CPEF02

The optimal culture medium for the marine fungal endophytic isolate CPEF02's growth and extracellular pigment was determined using five different growth media. The production of mycelial biomass ranged between 0.62 ± 0.05 and 2.43 ± 0.05 g/L. Maximum biomass was observed in PDB, by YEPD, SDB, MEB and CDB, respectively (Figure 2A). Among the six media tested, CDB, MEB, PDB, SDB and YEPDB, potato dextrose broth (PDB) was found to be the optimal media based on the maximum extracellular pigment production and absorption at 494 nm (λ_{max} for commercial Carmine standard) and absorbance unit (AU) at 490 nm/mL (Figure 2E). The yield of extracellular polyketide red pigment ranged from 1.59 ± 0.04 to 369.38 ± 0.53 mg eq. of Carmine per litre (Figure 2B). PDB media yielded the maximum extracellular red pigment followed by YEPD, SDB, GYEM, MEB and CDB, respectively (Figure 2B,C). The increased pigment production in PDB is partly due to the complex source of carbon in it [11,16]. In the solvent extracts, maximum pigment yield was observed in MSM obtained from Dianion HP 20 (647.87 mg. equivalent of Carmine per litre and AU_{490} 3.502) compared to MSE (107.85 mg. equivalent of Carmine per litre and AU_{490} 0.583). It was previously reported [17] that similar yields could be achieved by utilizing demineralized whey for the production of red extracellular pigment in *Monascus*. *Monascus* pigments are polyketide and hydrophilic in nature, and thus, methanol, being a more polar solvent than ethyl acetate, yielded the maximum red pigment. Previous studies [18,19] also found similar results and deemed non-polar solvents as not suitable for *Monascus* pigment extraction as they yielded less extracellular red pigment when compared with methanol.



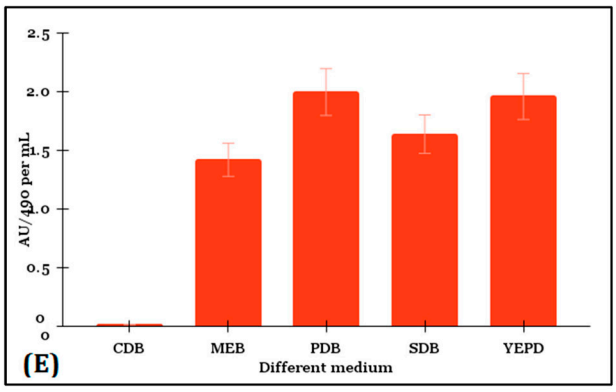


Figure 2. (**A**) Mycelial biomass of CPEF02 produced in different media, Czapek's extract dox Broth (CDB), malt extract broth (MEB), potato dextrose broth (PDB), Sabouraud dextrose broth (SDB), and yeast extract peptone dextrose broth (YEPD); (**B**,**D**) extracellular pigment production by CPEF02 (measured in mg equivalent of Carmine per litre) in different media; and (**C**) UV—visible absorption spectra of different media culture filtrates. (**E**) $AU_{490/mL}$ (absorbance unit at 490 nm/mL) of colourant produced in different media.

3.3. Antimicrobial Activity of CPEF02 Extracts

Antibacterial activity of CPEF02 extracts carried out using a disc diffusion assay showed notable inhibition zones (in mm) against four bacterial pathogens, namely, *S. aureus*, *S. typhimurium*, methicillin-resistant *S. aureus* and *V. cholerae* (Figure 3A–C, Tables 1 and 2). The maximum activity was present against *V. cholerae*, followed by *S. aureus* and then *S. typhimurium*. MSE exhibited maximum antibacterial activity followed by CFDP and methanol extracted colourant (MSM) (Table 1). The maximum inhibition activity of MSE was against *S. aureus*, followed by *S. typhimurium* and *V. cholerae.* As MSE had the highest level of extracellular pigment, its extract was also tested against the bacterial pathogens at multiple concentrations (100 µg/mL, 200 µg/mL and 400 µg/mL) (Table 2), and inhibitory activity was present at the lowest concentration, i.e., 100 µg/mL. *M. purpureus* extracts were tested previously by [20,21] for their antibacterial activities against *Bacillus subtilis* and *S. aureus*. These extract of CPEF02 exhibited some antibacterial activity, but this was lower than that observed for other strains (e.g., CPEF04 [10]). Antimi-

crobial activity may not be the primary activity of this extract, and further work is required to determine if this is a useful activity, including examining the potential mechanisms against both Gram-positive and Gram-negative bacteria.

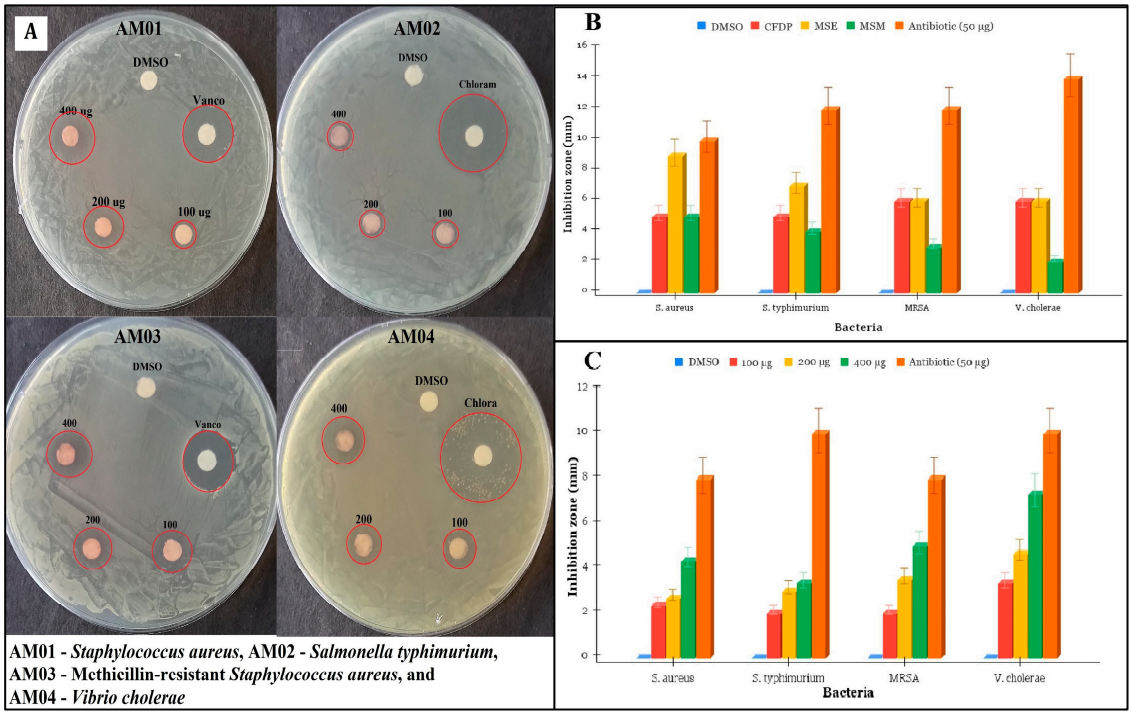


Figure 3. (**A**) Disc diffusion assay of CPEF02 (inhibition zone measured in mm); (**B**) antibacterial activity of CPEF02 extracts (CFDP, MSE and MSM) at the concentration of 1 mg/mL; (**C**) antibacterial activity of CPEF02 methanolic extract (MSM) at different concentrations against bacterial pathogens *S. aureus* (AM01), *S. typhimurium* (AM02), methicillin-resistant *S. aureus* (AM03) and *V. cholerae* (AM04).

Table 1. Antibacterial activity of CPEF02 extracts CFDP, MSE and MSM at the concentration of 1 mg/mL against bacterial pathogens (measured in mm).

	***S. aureus* (in mm)**	***S. typhimurium* (in mm)**	**MRSA (in mm)**	***V. cholerae* (in mm)**
DMSO	0	0	0	0
CFDP	5.77 ± 0.18	5.62 ± 0.08	6.7 ± 0.12	6.14 ± 0.9
MSE	9.33 ± 0.33	7.68 ± 0.2	6.42 ± 0.15	6.9 ± 0.33
MSM	5.45 ± 0.05	4.33 ± 0.18	3 ± 0.67	2.78 ± 0.05
Antibiotic (50 µg)	10	12	12	14

Table 2. Antibacterial activity (zone of inhibition) of CPEF02 methanolic colourant against bacterial pathogens (measured in mm).

	***S. aureus* (in mm)**	***S. typhimurium* (in mm)**	**MRSA (in mm)**	***V. cholerae* (in mm)**
DMSO	0	0	0	0
100 µg	2.33 ± 0.57	2 ± 0.00	2 ± 0.05	3.33 ± 0.66
200 µg	2.67 ± 0.35	3 ± 0.33	3.5 ± 0.33	4.67 ± 0.57
400 µg	4.33 ± 0.2	3.33 ± 0.57	5 ± 0.45	7.33
Antibiotic (50 µg)	8	10	8	10

Antifungal activity of CPPEF02 against the phytopathogens was tested by two methods—dual culture assay and another previously described method [13]. Antagonistic activity of the fungus was prominent against *A. solani* and *R. solani* with more than 50% inhibition, followed by *F. oxysporum* and *C. capsici* (Figure 4A–C). Antifungal activity of MSE was

maximal with 100% inhibition against *R. solani*, 84% inhibition against *A. solani*, 72% against *F. oxysporum* and 37% against *C. capsici* (Figure 4E). MSM (Figure 4F) and CFDP (Figure 4D) showed similar activity of 50% inhibition against *R. solani*, 31% against *F. oxysporum* and none against *C. capsici.* MSM had better (15%) activity against *A. solani* as compared with CFDP (7%). Due to the medium polarity of the solvent (ethyl acetate), the MSE extract may contain non-polar metabolites of antimicrobial efficacy. Purpureusone is a known antifungal pigmented metabolite that is found in *M. purpureus*, as identified by [22], and inhibits the growth of *Candida albicans* and *Streptomyces cerevisiae*. Furthermore, the antibacterial and antifungal activity of the *Monascus purpureus* colourant is similar to ones reported [21,23]. The antibacterial activity of the colourant isolated from *M. purpureus* has received less attention than the antimicrobial activity of fungal fermented rice powder or *Monascus* koji powder. The current work sought to shed light on the bioactivity of the *M. purpureus* colourant [24].

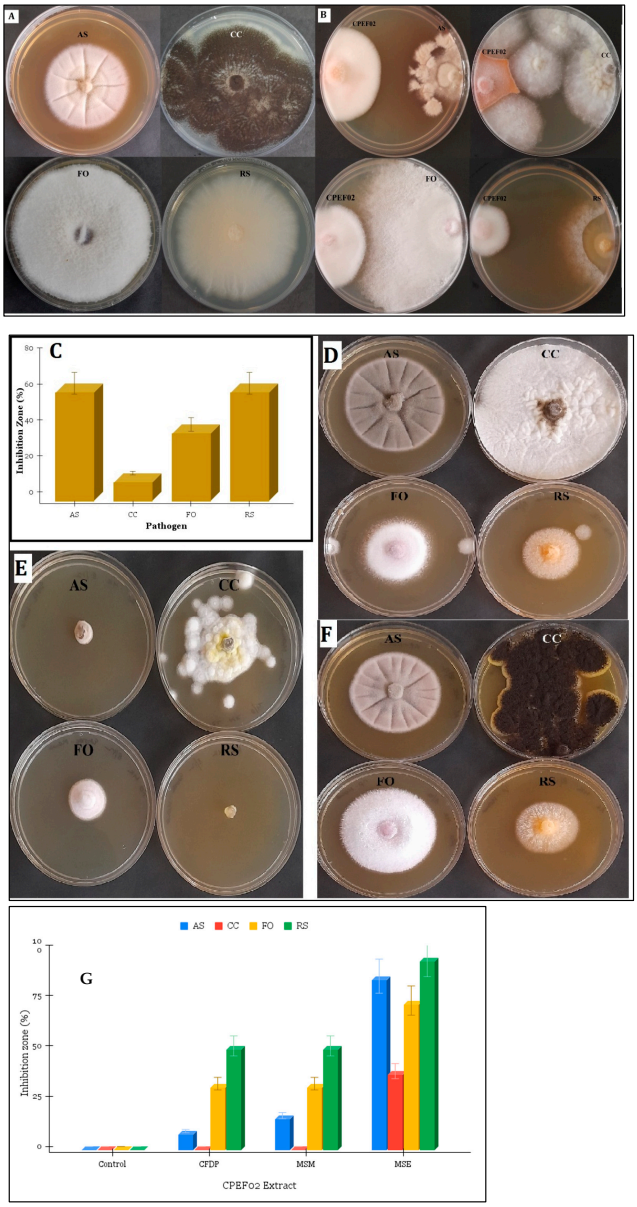


Figure 4. (**A**–**C**) Antagonistic activity of CPEF02 on pathogenic fungi *A. solani* (AS), *C. capsici* (CC), *F. oxysporum* (FO) and *R. solani* (RS). (**D**) Antifungal activity of CFDP, (**E**) antifungal activity of MSE extract, (**F**) antifungal activity of MSM extract and (**G**) inhibition zone in percentage (%).

3.4. Antioxidant Activity of CPEF02 Colourant

As MSM gave the highest extracellular polyketide pigment output, its antioxidant efficiency was also determined. The antioxidant activity of the CPEF02 methanolic colourant (MSM) was estimated with the help of ABTS radical scavenging assay by calculating IC_{50} (sample concentration to scavenge 50% ABTS radicals) and Trolox equivalent antioxidant capacity (TEAC). Ascorbic acid, TBHQ and Trolox were used as positive standards (Figure 5). The IC_{50} value of the CPEF02 colourant (MSM) against these radicals was found to be 14.42 µg/mL, while the TEAC value was 0.571 µM Trolox/µg of the pigmented extract. These results indicated a higher antioxidant activity against ABTS radicals and were consistent with the antioxidant activity shown against ABTS radicals [21,25]. *Monascus* pigments such as rubropunctamine and monasfluore B are known antioxidant compounds [26,27].

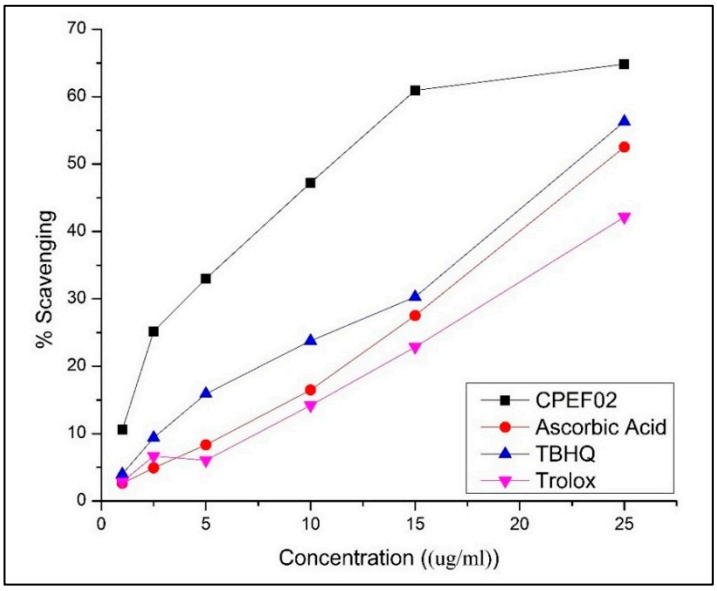


Figure 5. Antioxidant activity of methanolic CPEF02 extract and standards (ascorbic acid, TBHQ and trolox) against the ABTS radical.

3.5. LC-HRMS Profiling of CPEF02 Pigments

Colourant profiling and chemical characterization were performed after subjecting the methanolic extract (MSM) to LC-HRMS (Figure 6 and Table 3). The total ion chromatogram (TIC) (Figure 6A) and a table of the identified compounds by the molecular ion's $[M + H]^+$ mass/charge ratio (Table 3 and Figure 6B) with their structures are shown. The chromatogram showed a high intensity peak at retention time (R_t) 10.965 with a mass by charge ratio (m/z) of 385.2. This peak was identified as Monasfluore B with a molecular formula of $C_{23}H_{28}O_5$ (Figure 6C) [28]. Monasfluore B is known for its antioxidant activity tested in mice models [27]. Other pigmented compounds identified were rubropunctine [29], PP-V [30], monaphilones B and C [31], purpureusone [22], monascin and rubropunctamine [5,32]. *Monascus* pigments are known for their antimicrobial and antioxidant activities, and their presence enhances the pharmaceutical potential of this material. Monascin and ankaflavin are known for their anticancer, antidiabetic, antioxidant, anti-obesity and anti-inflammatory properties and are relatively well studied pigments along with Monacolin K [33–38]. Monasfluore A and B have been tested on mice models and were found to exhibit antioxidant and anti-obesity activities [27]. The major pigments reported from *Monascus purpureus* strains are monascin (yellow); monascorubrin (orange); monascorubramine; monapurone A–C; monasphilone A, B and C; monasfluore A and B; monascolin K; ankaflavin; rubropunctamine; rubropunctatin; and monopilol A–D [7,28,31,39–42]. However, only a few of these, namely, monascin, ankaflavin, rubropunctatin and monascorubamine, are used in the food and dyeing industries [7,43]. The

extract's antioxidant action may be ascribed to monasfluore B, monascin and rubropunctamine, while its antibacterial activity may be related to purpureosone. These compounds identified in the methanolic extract are most likely responsible for the antibacterial and antioxidant properties of the extracts.

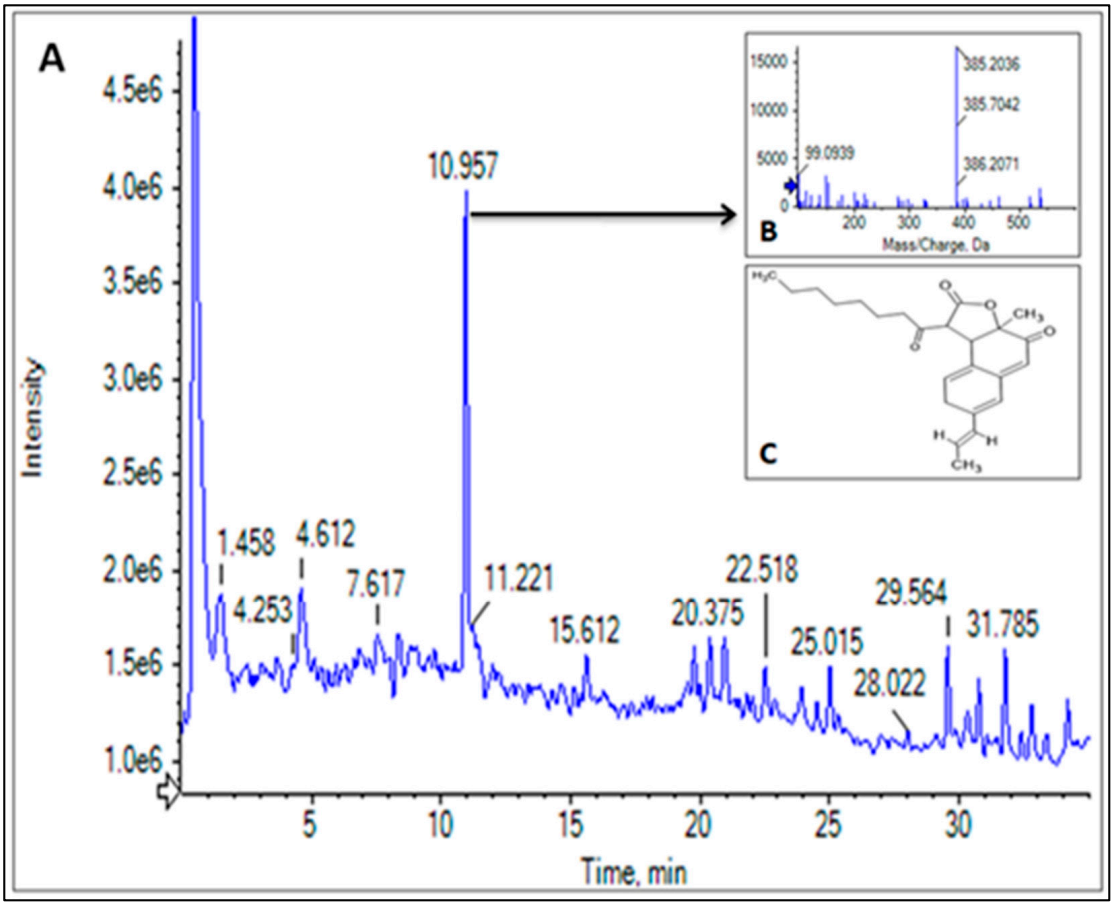


Figure 6. (**A**) Liquid chromatogram of methanolic extract of CPEF02, the arrow on the y-axis indicating base (starting point) for the intensity, (**B**) mass spectra of Monasfluore B at retention time 10.965, (**C**) molecular structure of Monasfluore B.

Table 3. Pigmented compounds in the methanol-extracted CPEF02 colourant determined using LC-HRMS analysis.

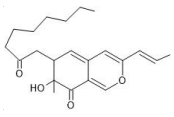
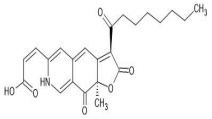
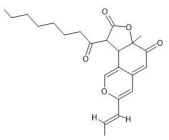
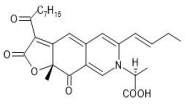
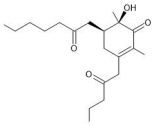
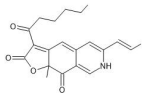
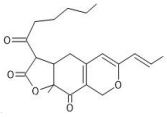
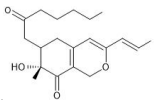
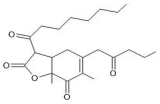
Analyte No.	Tentative Compound Allotted	Chemical Formula	Retention Time	Structure	Mass Recorded (*m*/*z*)	Activity	References
1	Rubropunctin	$C_{22}H_{30}O_4$	4.610		359.67	-	[29]
2	PP-V (*Penicillium purpurogenum*–Violet)	$C_{23}H_{25}NO_6$	8.361		411.66	-	[30]
3	Monasfluore B	$C_{23}H_{28}O_5$	10.965		385.20	Antioxidant, anti-inflammatory activity	[5,27,28]

Table 3. *Cont.*

Analyte No.	Tentative Compound Allotted	Chemical Formula	Retention Time	Structure	Mass Recorded (*m*/*z*)	Activity	References
4	Red derivative 1	$C_{26}H_{31}NO_6$	18.173		453.34	-	[44]
5	Monaphilone C	$C_{20}H_{32}O_4$	22.019		337.241	Anti-proliferative	[31]
6	Rubaropunctamine	$C_{21}H_{23}NO_4$	23.922		354.25	Antioxidant, anticancer activity	[5,45,46]
7	Monascin	$C_{21}H_{26}O_5$	24.518		359.18	Anticancer, antitumor, antidiabetic, antioxidant, anti-inflammatory, anti-obesity activity	[5,33–38]
8	Monaphilone B	$C_{20}H_{28}O_4$	25.028		333.21	Anti-proliferative activity	[31]
9	Purpureusone	$C_{23}H_{34}O_5$	25.349		391.21	Antifungal activity	[22]

4. Conclusions

Microbial pigments can be good alternatives to synthetic and plant-based pigments because of their stability, bioactivity and cost-effective production. Apart from adding colour, they increase the health potential of food with bioactivities such as antibacterial, antifungal and antioxidant activities. This work investigated the optimal media and extraction conditions to obtain maximum extracellular polyketide pigment from the endophytic marine fungus *Monascus purpureus* CPEF02. The methanolic extract of the fungus was found to produce maximum extracellular pigment (647.87 mg. equivalent of Carmine per litre) along with exhibiting antibacterial and antioxidant activities. The antioxidant activity of 14.42 µg/mL IC_{50} and a TEAC value of 0.571 µM Trolox/µg was also obtained for the pigmented extract. The ethyl acetate extract was shown to have antimicrobial activity and inhibited the growth of both bacterial and fungal pathogens (more than 90% inhibition in the case of fungal pathogens *R. solani* and *A. solani* and 9.33 ± 0.33 mm zone of inhibition in the case of bacterial pathogen *S. aureus*). The presence of Monasfluore B, monascin, purpureosone and rubropunctamine in the methanolic extract of CPEF02 is consistent with its observed activities. CPEF02 was shown to have higher organic solvent solubility and higher antioxidant activity but lower antibacterial activity than pigment from a previous fungal strain (*Talaromyces* strain CPEF04 [10]). CPEF02 is derived from red yeast rice, while CPEF04 is from a non-food grade strain, and so, the pigment reported here has more potential to be developed into a food grade product. These pigments could be further developed for pharmaceutical, food, cosmeceutical, or sensitized solar cells or textile applications, particularly where their bioactivity could be used as an additional benefit to their colour. The biosynthetic pathways of the pigments being produced could be systematically explored for biotransformation of the *Monascus* cells into cell factories via metabolic engineering for industrial-scale production. Pigment production could be readily scaled to the pilot and manufacturing scales and formulated for pharmaceutical, textile or food use.

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