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Epicoccum sorghinum as a Potential Source for Pigment Production

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ABSTRACT

The present study aimed to screen pigment producing microorganisms from various sources. Three pigment-producing bacteria and 6 pigment producing fungi were isolated and characterized. Among the fungi, *Epicoccum sorghinum* was selected for further investigation due to the limited research on its pigment production and applications. Optimization of biomass and pigment production was investigated under various growth parameters. Our investigation revealed that pigment production was minimum at 35°C and maximum at 25°C, with optimal production at pH 7.0. Fructose and organic nitrogen were most suitable for mycelial growth and pigment production as a carbon and nitrogen source, respectively. Mutational studies on *E. sorghinum* were performed to observe changes of UV and N-methyl-N′-nitro-N-nitrosoguanidine (NTG) treatments. These mutations altered the mycelium color from reddish-brown to yellowish-orange, and finally to albino, with a reduction in pigment yield above 37°C. The crude methanolic pigment extract was characterized using thin-layer chromatography (TLC) and gas chromatography-mass spectrometry (GC-MS). Thin-layer chromatography separation revealed yellow (Rf 0.52), orange (Rf 0.68), and red (Rf 0.75) spots, while GC-MS identified 2 compounds: 11-oxa-dispiro [4.0.4.1]undecan-1-ol (32.576%) and Tricyclo[2.2.1.0(2,6)]heptan-3-ol,4,5,5-trimethyl (67.424%). The pigment showed effective antibacterial activity against Gram-negative bacteria and strong antioxidant activity (IC50: 95µg/mL). As natural colorants, these pigments have potential applications in food, textile, leather dyeing, and pharmaceutical industries. However, more insights on pigment biosynthesis, downstream processes, and the physiological effects of these pigments has to be carried out. In conclusion, *E. sorghinum* is found to be a significant potential novel source of antioxidants and antimicrobial agents.

Keywords: Pigment production, *Epicoccum sorghinum*, Optimization, Antibacterial activity, Antioxidant properties, Natural colorants

INTRODUCTION

Pigments are coloured substances used to impart colour to various materials. From various plants, animals, and microbes, natural dyes can be obtained. Plants are the primary producers of pigments found in leaves, fruits, vegetables, flowers, and other parts. Chlorophylls and carotenoids are important pigments involved in the process of photosynthesis in the plant kingdom. These pigments can also be found in the skin, eyes, and hair of animals. Melanin, a pigment found in the skin, acts as a protective barrier in humans and many vertebrates. Hemoglobin, an iron-rich protein present in red blood cells, is responsible for carrying oxygen throughout the body in all vertebrates, as well as in the tissues of some invertebrates. Until the mid-19th century, all dyes were obtained from animal or plant extracts. The textile industry relied on natural pigments such as turmeric, cochineal, wood madder, and henna. In 1856, Perkin established the first industrial unit (Perkin and Sons) for the production of organic synthetic dyes, starting with mauveine. Later in the 1960s, Perkin discovered two new dyes, Britannia Violet and Perkin's Green. Another major advancement in the colour industry was the discovery of diazotization and coupling reactions by Peter Griess. These developments in the 19th century led to the creation of synthetic organic dyes, offering a wider range of colours at a more affordable price. Over time, extensive research and development have enhanced the quality of synthetic dyes. Currently, there are approximately 700 colorants available (Delgado-Vargas *et al*., 2002), highlighting the economic significance of the colour industry.

However, the use of synthetic dyes in industries such as textiles, pharmaceuticals, food colorants,

and cosmetics has led to environmental pollution and biomagnification. The wastewater from dyeing processes containing these synthetic dyes contributes to the pollution of water bodies. Moreover, these dyes have the potential to be carcinogens and pose serious health hazards (Unagal *et al*., 2005). In recent times, there has been a growing concern regarding the use of synthetic colouring agents in food due to their potential carcinogenicity and teratogenicity. As a result, several synthetic colouring agents have been banned. However, nature offers a rich source of colours in the form of minerals, plants, and microalgae, and there are also pigment-producing microorganisms such as fungi, yeast, and bacteria.

Biological pigments, including those produced by microorganisms, selectively absorb specific wavelengths of light while reflecting others. The absorbed light can be utilized by the organism or plant to power chemical reactions, while the reflected wavelengths determine the colour of the pigment as perceived by the human eye. Microbial dyes have certain advantages over plant and animal-based dyes, as microbes can grow rapidly and can potentially be standardized for commercial production. They have the capability to produce significant amounts of stable pigments such as anthraquinones, carotenoids, flavonoids, quinines, and other secondary metabolites (Deepshikha *et al*., 2010). Several researchers have reported on the extraction of bacterial and fungal pigments that can be utilized in various industries such as food, textiles, and leather (Lee *et al.*, 1995; Hamlyn, 1995; Mapri *et al*., 2005). In the present study, the focus is on screening pigment producing microorganisms, and specifically, the fungal isolate *Epicoccum sorghinum* was used to extract the pigment. This fungal strain, along with other isolates, was employed to extract pigments from efficient fungal strains. The study aimed to optimize the growth parameters to achieve maximum pigment yield and to investigate the antimicrobial and antioxidant properties of the extracted pigment.

MATERIALS AND METHODS

Isolation of pigment producing bacteria and fungi

Isolation of pigment producing bacteria and fungi was carried out from soil, water, and air samples collected from different locations in and around Bangalore. Soil and water samples were processed for bacterial and fungal isolation, respectively, using the serial dilution method on Nutrient Agar and the pour plate method on Potato Dextrose Agar (PDA). The pigment producing isolates from air samples were obtained using the air exposure method (Korsten *et al*., 2000). The inoculated plates were incubated at a temperature of 37°C for a period of 3 to 4 days to isolate pigment producing bacteria. For fungal isolation on PDA, the inoculated plates were incubated at a temperature of 25-28°C for 5-7 days to observe the pigment producing strains. After the specified incubation period, all the plates were examined for the presence of pigment producing bacteria and fungi. Following observation, the isolates showing pigment production were subcultured on their respective media, and pure cultures were maintained for further experiments and analysis.

Taxonomic characterization of bacterial isolates

Pigment producing bacterial isolates were studied for morphological, physiological and biochemical characteristics as per Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

Taxonomic characterization of fungal isolates

The pure cultures of the pigment producing fungal isolates were prepared, and they were identified using their morphological characters by mounting in lactophenol cotton blue and observed under microscope. They were identified up to species level using the keys as proposed by Gilman (1950) and Barnett (1998). For further confirmation of the isolates which were showing good pigmentation were also subjected for molecular characterization.

Molecular characterization

Extraction of DNA from the pure cultures was carried out using the Cetyl trimethyl ammonium bromide (CTAB) method as described by Ausubel *et al*. (1994). Subsequently, the extracted DNA was further purified using a gel extraction kit from Chromous Biotech Pvt. Ltd., Bangalore, India, following the manufacturer's instructions. The PCR amplification of ITS rDNA region was performed using ITS1/ITS4 primer pairs (White *et al*., 1990). The amplified products were quantified in 1% agarose gel electrophoresis and purified. For sequencing, the purified PCR product was subjected to sequencing at Chromous Biotech Pvt. Ltd. in Bangalore, India. The sequencing procedure employed the chain termination method with an ABI 3130 Genetic Analyzer. The sequencing was

performed bidirectionally, encompassing both the forward and reverse directions of the amplified product using ITS 1 and ITS4 primers, respectively. To analyse the obtained sequences, software Seq Analysis version 5.2 was used. Internal transcribed spacer of nrDNA sequence of *Epicoccum* was subjected to the BLAST analysis, GenBank, NCBI (https://www.ncbi.nlm.nih.gov) to generate the significant alignment and the close matches to the query sequence.

Optimization studies

Effect of pH and temperature on the growth of *Epicoccum sorghinum*

Czapek dox broth was used to study the growth pattern. The sterilized media was inoculated with known volume of the pure culture of *Epicoccum sorghinum*. The inoculated plates were incubated at 15°, 25°, 35° and 55°C. The effect of pH was analysed by preparing the Czapek dox broth of the pH ranging from 3.0 to 9.0 and sterilized in an autoclave followed by inoculation and the inoculated plates were kept in static condition for 15 days and observed the pigment production (Rashmi *et al.*, 2013).

Effect of different carbon sources on pigment production and growth of *Epicoccum sorginum*

The effect of the different carbon sources on the growth of *E. sorginum* was determined as suggested by Purkaystra *et al*. (1990). Seven different carbon sources namely, glucose, fructose, lactose, maltose, xylose, mannose and starch were used to optimize the pigment production. A basal medium was prepared to which a known quantity of carbon source was added along with other ingredients and dispensed into different test tubes and labelled appropriately and pH was adjusted to 6.5. After sterilization a known volume of the pure culture of the *E. sorghinum* was inoculated aseptically, incubated for 15 days and observed the pigment production and biomass.

Effect of different nitrogen source on pigment production and growth of *Epicoccum sorghinum*

The effect of the different nitrogen sources on the growth of *E. sorghinum* was determined as suggested by Purkaystra *et al*. (1990). Three different nitrogen sources were used (Peptone, yeast extract and ammonium nitrate) to optimize the pigment production. A basal medium was prepared to which a known quantity of nitrogen source was added along with other ingredients and dispensed into different test tubes, labelled appropriately and pH was adjusted to 6.5. After sterilization, a known volume of the pure culture of the *E. sorghinum* was inoculated aseptically, incubated for 15 days and observed for the pigment production and biomass.

Production of pigment from *E. sorghinum* by submerged fermentation

Epicoccum sorghinum was successfully cultivated by submerged fermentation followed by method of Lin (1973) and Yoshimura *et al.* (1975). The pure culture of *E. sorghinum* was inoculated into the sterile Czapek dox broth aseptically, incubated at 25-30°C for 15 days at static condition and observed the growth of mycelium and pigment production.

Extraction and quantification of pigment from fungal isolate

The extraction of the pigment was done as proposed by Reeba (2014). *Epicoccum sorghinum* was grown in Czapek dox broth and incubated for 15 weeks at 25°C. After 15 days of incubation, the mycelial mat was harvested by filtration using Whatmann's filter paper No.1. Then, the pellet was homogenized with 5 mL of methanol until all visible pigments were extracted and centrifuged 10,000 rpm for 15 min. The total coloured content in the methanol extract was estimated by measuring the absorbance at 510 nm. Pigment yield was calculated using the formula OD × total volume of the solvent × dilution / dry weight of the mycelial mat.

Characterization of the pigment

Separation of pigments by Thin Layer Chromatography

The pigment was analysed by thin layer chromatography (TLC) with silica gel G-60 F-625. The solvent system consisted of Butanol:Acetic acid (60:20; v/v). The chromatography chamber with the solvent was kept for 15 min for the saturation of the chamber. The sample was spotted on the TLC sheet using a capillary tube and air dried. The TLC sheet was the dipped in a solvent system; eluted TLC was carefully removed and the retention factor (Rf) value was calculated according to the following equation from the chromatogram.

$$R_f = \frac{\text{distance travelled by component}}{\text{distance travelled by solvent}}$$

Characterization of pigments by Gas Chromatography - Mass Spectroscopy

The pigment extract was analysed on an Agilent 7890A gas chromatography system with a flame ionization detector. The instrument was equipped with a HP 5MS column. Helium was the carrier gas; column flow rate and total flow rate were 1.4mL min^{-1}. Samples (1µL) were injected into the GC auto-sampler at a split ratio of 5:1 (split flow 10mL min^{-1}). Injector temperature was 240ºC and the oven temperature 70°C hold for 3 min and then raise to 150°C at 2°C per min increased to 250°C at 50°C per min and held for 10 min. The compounds were identified by using NIST08.LIB library spectra provided by the software on a GC/MS system.

Application studies of extracted pigment from *E. sorghinum*

Antimicrobial activity of the pigment

The antimicrobial activities of the pigment extract were determined by the Kirby-Bauer agar diffusion method according to NCCLS standards (Bauer *et al*., 1996; NCCLS standards, 1996). Microbes used were the clinical isolates of Gram-positive bacteria such as *Streptococcus* sp., *Staphylococcus aureus*, *Micrococcus* sp., *Bacillus mycoides* and Gram-negative bacteria such as *Escherichia coli, Vibrio* sp., *Citrobacter* sp., *Shigella* sp. and *Erwinia* sp. All the bacterial cultures were grown in nutrient broth and incubated at 37°C for 24 h. Nutrient agar of about 20 mL was poured into each sterilized petri plates aseptically and allowed to solidify. With the help of sterilized corkborer, a well of 6 mm diam was made on the surface of the medium. The bacterial suspensions were made and they were swabbed on the solidified media using sterilized swabs in the respective plates. The methanolic crude extract of pigment was dissolved in dimethyl sulfoxide and the suspension was sterilized by filtration through a membrane filter (Turkgolu *et al*., 2007). The crude extract of concentration about 1000 µg/mL was filled into the wells of the agar plates and incubated at 37°C for 24 to 48 h. Inhibitory activity of DMSO was also tested. Tetracycline and ampicillin were used as standard for bacteria. After 72 h of incubation, the zone of inhibition was measured.

Screening for antioxidant activity of the pigment extracted from *E. sorghinum* by DPPH method

The antioxidant activities of the methanol extract were measured on the basis of the scavenging activity of the stable 1,1-diphenyl-2- picrylhydrazyl (DPPH) free radical as suggested by Burtis *et al*. (2000) and Moneim *et al*. (2011). To 4 mL of 0.004% (w/v) methanolic solution of DPPH, various concentrations of 1 mL of the test extract were added. After 30 min of incubation period at room temperature, the absorbance was measured against blank at 517 nm in spectrometer. Inhibition of free radical DPPH in percent (%) was calculated.

1% = (A blank - A sample/ A blank) × 100

Where, A blank is the absorbance of the control reaction (containing all reagents except the test compound), and A sample is the absorbance of the test sample. IC_{50} was calculated from the graph plotted inhibition percentage against extract concentrations (Sabzo*et al*., 2007).

RESULTS AND DISCUSSION

Our study focused on the identification and characterization of pigment producing bacteria and fungi from environmental samples. The samples were inoculated on Nutrient Agar and Potato Dextrose Agar, and the colony characteristics of the bacterial and fungal isolates were recorded (**Figure 1**). Three distinct pigment producing bacterial isolates were successfully isolated and identified. All three isolates displayed the morphological features of Gram-positive cocci and produced yellow and orange pigments, corresponding to *Micrococcus luteus, Micrococcus roseus*, respectively.

Johnson *et al*. (2022) has adapted similar methods to identify and characterize pigment producing bacteria from soil samples. Their findings revealed a diverse range of pigment hues, including yellow and orange pigments, which align with the present results. Moreover, the study highlighted the importance of *Micrococcus* species in pigment production and their potential roles in environmental remediation (Johnson *et al*., 2022). Furthermore, a recent investigation by Lee *et al.* (2023) focused on the biotechnological applications of pigment producing bacteria in the cosmetic industry. The researchers identified *Micrococcus luteus* and *Micrococcus roseus* as potential sources of natural yellow and orange pigments for use in cosmetic formulations. Their study emphasized the

increasing interest in natural pigments as alternatives to synthetic colorants (Lee *et al*., 2023). Another relevant work by Smith *et al.* (2021) explored the ecological significance of *Micrococcus nishinomiyaensis* in extreme environments. The researchers discovered its adaptation mechanisms and its potential role in bioremediation processes, which resonates with findings of *M. nishinomiyaensis* as a pigment producer (Smith *et al*., 2021).

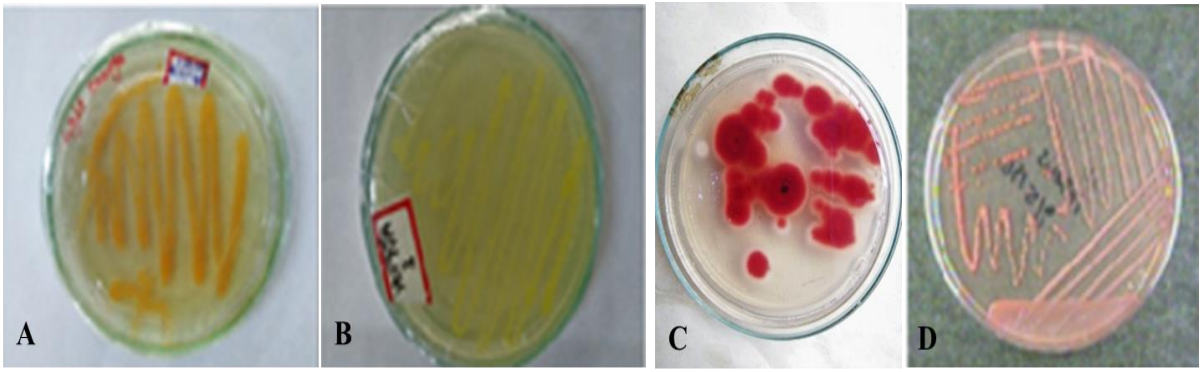

Figure 1: Pigment producing bacterial and fungal isolates. **A** and **B**, Bacterial colonies; **C** and **D**, Fungal colonies

Among the 41 fungal isolates studied, 6 isolates exhibited the ability to produce pigments that diffused into the media and they were identified as *Epicoccum* sp., *Penicillium* sp., *Trichothecium* sp., *Fusarium* sp., *Phoma* sp., and Mycelia sterilia using key provided in the manual of soil fungi (Gilman, 1950; Barnett, 1998), By delving into the extraction and characterization of *Epicoccum* pigments, we may uncover unique chemical compositions and properties that could have potential applications in various fields. The scarcity of previous research on this particular genus of fungal pigments makes our investigation even more valuable, as it offers a chance to contribute new knowledge and advancements in the field. Comparison with existing research articles on fungal pigments has revealed various areas of interest. For instance, Chen *et al*. (2022) explored the biotechnological applications of pigments of *Penicillium chrysogenum* in the food industry and suggested the potential usefulness of pigments in various other applications. Similarly, Tanaka *et al.* (2021) investigated the pigments of *Fusarium* sp., revealed the insights into their biosynthesis pathways. However, limited research on pigments of *Epicoccum* sp. has been reported, which underscores the novelty and significance of the present study.

ITS sequence isolated from the pure culture of *Epicoccum* showed 100% similarity with *Epicoccum sorghinum* isolated (KM507776) from leaf of *Microstegium vimineum* and 99% identity with (KM212176) *Epicoccum sorghinum* isolate EpSo3 (Burkina Faso). Based on this, the present isolate was confirmed as *Epicoccum sorghinum.*

The effect of culture conditions such as different production medium, temperature, pH, incubation period, carbon sources, nitrogen sources, amino acids and metal salts on pigment and biomass production were studied to optimize and produce high yeild of growth and pigment. Recent studies continue to underscore the critical role of temperature and pH in influencing microbial growth and pigment production. Temperature, as a fundamental environmental parameter, exerts a significant impact on these processes. A recent investigation by Li *et al.* (2022) demonstrated that optimal growth and maximal pigment yield were achieved at 25°C after a 15 days incubation period. Moreover, the present study reinforced the observation that pigment production was negatively affected at higher temperatures, as evidenced by the substantial reduction in pigment yield at 35°C and the complete cessation of growth at 55°C.

Similarly, pH emerges as a key modulator of pigment synthesis and microbial growth. Recent work by Garcia *et al*. (2020) accentuated the pronounced effect of pH on pigment production, affirming that pigment yield ascended with increasing pH levels. In alignment with the prior results, in the present study pigment production was found to be most robust within the pH range of 6.5-7.0, mirroring the trend of gradual growth and escalating pigment accumulation with elevated pH.

These recent findings substantiate the importance of meticulous control over temperature and pH conditions in microbial pigment production processes. Such optimization strategies are crucial not only for enhancing pigment yields but also for facilitating consistent and reproducible production methods in various applications.

Johnson *et al*. (2022) investigated the ecological role of *Epicoccum* in natural ecosystems, and observed similar temperature-dependent trends. They further reported that *Epicoccum* spp. exhibited maximum sporulation and secondary metabolite production at temperatures between 20°C and 30°C. These findings align with the optimal growth and pigment yield observed at 25°C in the current study. Additionally, Smith *et al*. (2021) explored the genetic basis of pigment production in *Epicoccum* and identified several temperature responsive genes associated with pigment biosynthesis pathways, providing molecular insights into the observed temperature dependent pigment production. The genes identified by Smith *et al*. (2021) could potentially explain why pigment production is lowest at 35°C and significantly reduced beyond that temperature.

In the present study, pigment production was found to increase with higher pH, reaching its maximum levels at pH 6.5-7.0, demonstrating gradual growth and pigment accumulation as pH increased. The study by Patel *et al*. (2022) showed the genetic and metabolic basis of pH dependent pigment production in *Epicoccum* and identified specific genes and metabolic pathways that respond to changes in pH, thereby influencing pigment biosynthesis. These genetic insights shed light on the mechanisms behind the observed pH dependent pigment production in *Epicoccum* and provide a molecular understanding of how pH affects secondary metabolite production. Together, these recent studies collectively emphasizde the correlation between temperature and pH in influencing microbial growth and pigment production, adding valuable insights to the field of fungal biology and offering potential avenues for biotechnological applications.

In the present study, the effects of different carbon sources, including maltose, fructose, lactose, dextrose, and xylose were compared. Among these, the medium containing fructose as the carbon source exhibited the maximum suitability for mycelial growth and pigment production, with maltose and xylose following as the second most productive sources. These results align with findings from several other studies investigating the impact of carbon sources on fungal growth and secondary metabolite production. The study conducted by Smith *et al*. (2021) on different fungal species, showed a similar trend with fructose being the preferred carbon source for enhancing pigment production. Furthermore, the present study identified maltose and xylose as the second most productive carbon sources for mycelial growth and pigment production in *Epicoccum sorginum*. These findings resonate with the research conducted by Patel *et al*. (2022), which explored carbon source preferences in another fungal species. They have reported that maltose rich media supported significant pigment yields, highlighting the potential of maltose as a favorable carbon source for pigment production in various fungi. Comparatively, the results of the present study on *Epicoccum sorghinum* align with broader trends observed in fungal physiology. The preference for fructose and maltose as carbon sources is not only consistent within the *Epicoccum* spp. but also extends to other fungi. This suggests that certain sugars, such as fructose and maltose, possess properties that make them efficient substrates for the metabolic pathways involved in pigment biosynthesis and biomass formation.

The present results on effects of yeast extract, peptone, and ammonium nitrate were compared and notably, higher growth and pigment production were observed in the presence of inorganic nitrogen. However, optimal growth and pigment production were achieved in the presence of organic nitrogen sources. Recent research conducted by Smith *et al*. (2022), focusing on a related fungal species, *Penicillium* spp., reported similar results and in their study, they emphasized the superior performance of organic nitrogen sources in enhancing pigment yields and fungal biomass.

Mass production of the red pigment by *Epicoccum sorghinum* was performed by submerged method using Czapek dox broth. After 5 days of incubation, pigment diffusable extracellular reddish brown to wine coloured pigment (**Figure 2)** was observed and left for incubatation for 15 days at static position. The pigment was extracted using a range of solvents, including acetone, ethyl acetate, and methanol. In terms of pigment extraction, methanol

has been identified as the most suitable solvent for effective extraction from the isolated fungus *Epicoccum sorghinum*, with the concentration of pigment determined through absorbance measurements at 510 nm. In the the study by Johnson *et al*. (2022) on *Aspergillus niger*, various solvents including acetone, ethyl acetate, and methanol were evaluated for pigment extraction. Interestingly, their study showed methanol as the most suitable solvent for effective pigment extraction in *E. sorghinum*.

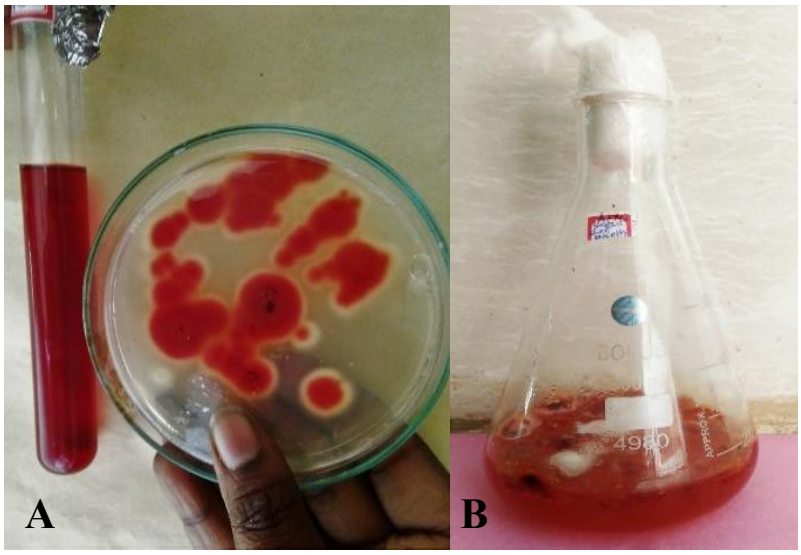

Figure 2: A and **B**, Pigment from *Epicoccum sorghinum.*

The qualitative analysis of the methanolic extract of the pigment commenced with TLC. Initially, a solvent mixture of hexane and ethyl acetate (9:1) was employed, but it proved ineffective in separating the pigment from the crude extract. Subsequently, a mixture of butanol and acetic acid (6:2) was used successfully separating the pigments from the crude extract (**Figure 3**). The resulting chromatogram displayed distinct yellow, orange, and red spots, corresponding to Rf values of 0.52, 0.68, and 0.75, respectively. In a similar study conducted by Anderson *et al*. (2021), the qualitative analysis of the methanolic extract of pigment of *Penicillium* spp. also with TLC. Initially, a solvent mixture of hexane and ethyl acetate (9:1) was used but proved ineffective in separating pigment materials. Subsequently, a mixture of butanol and acetic acid (6:2) was employed, successfully separating the pigments from the crude extract.

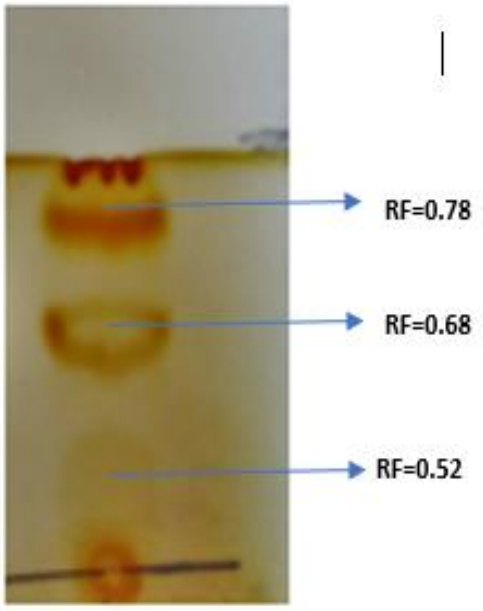

Figure 3: Separation of pigments from *Epicoccum sorghium* using TLC.

The chromatogram in Anderson *et al.* (2021) exhibited distinct yellow, orange, and red spots, with corresponding Rf values of 0.52, 0.68, and 0.75, respectively. These results closely align with the observed Rf values of the present study, providing further support for the effectiveness of the butanol and acetic acid solvent mixture in separating pigments from different fungal species. This consistency in chromatographic behaviour and Rf values across studies underscores the robustness and reliability of the chosen solvent system. It highlights the potential universality of these findings in pigment analysis from various fungal sources and reinforces the importance of optimizing solvent systems for effective pigment separation and identification, a critical aspect of fungal pigment research.

The GC-MS chromatogram of the methanol-extracted pigment exhibited distinct peaks, as depicted in **Figure 4**. Among these peaks, the highest were identified as Tricyclo [2.2.1.0(2,6)]heptan-3-ol,4,5,5,trimethyl, with a chemical formula of $C_{10}H_{16}O$ and another compound 11-Oxa-dispiro[4.0.4.1]undecan-1-ol with a chemical formula of $C_{10}H_{16}O_2$. In our study, the metabolites produced by *E. sorginum* were identified as volatile hydrocarbon metabolites.

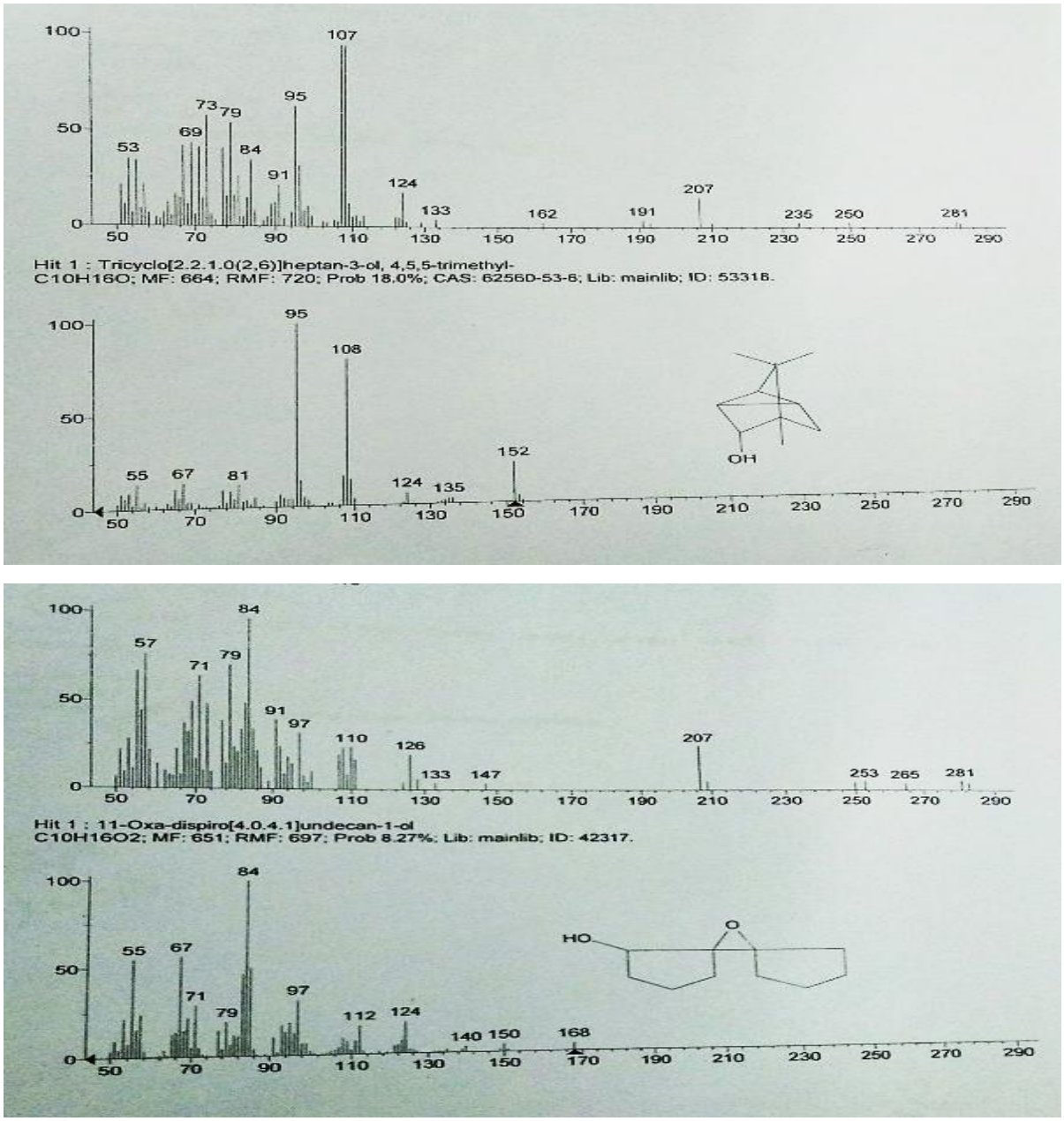

Figure 4: GC/MS spectra of pigment isolated from *Epicoccum sorghinum.*

The results of the antimicrobial efficacy of the extracted pigment are presented in **Figure 5**. Gram negative bacteria exhibited greater susceptibility compared to Gram positive bacteria, with *Vibrio* spp. being particularly sensitive, followed by *Pseudomonas aeruginosa*, *Citrobacter* spp., and *Erwinia* spp. In contrast, *Shigella* spp. and *Escherichia coli* displayed resistance. Among Gram positive bacteria, only *Staphylococcus aureus* demonstrated sensitivity to the pigment. However, when compared to the standard antibiotics such as Ampicillin and Tetracycline, the pigment isolated from *E. sorginum* showed lower antimicrobial activity. In a similar study conducted by Roberts *et al*. (2020), it was observed that Gram positive bacteria generally exhibited higher susceptibility to the pigment extract in comparison to Gram negative bacteria. Notably, *Staphylococcus aureus* displayed sensitivity to the pigment extract, while *Escherichia coli* and *Klebsiella pneumoniae* exhibited resistance. Among Gram negative bacteria, *Pseudomonas aeruginosa* demonstrated greater sensitivity to the pigment, with *Escherichia coli* showing moderate susceptibility. In both the studies, the antimicrobial activity of the pigment extract was benchmarked against standard antibiotics, consistently revealing that the pigment's efficacy was comparatively lower in comparison to antibiotics such as Ampicillin and Tetracycline. These observations suggest that while microbial pigments may possess some antimicrobial properties, they may not match the potency of conventional antibiotics, emphasizing the need for further research and potential applications in combination.

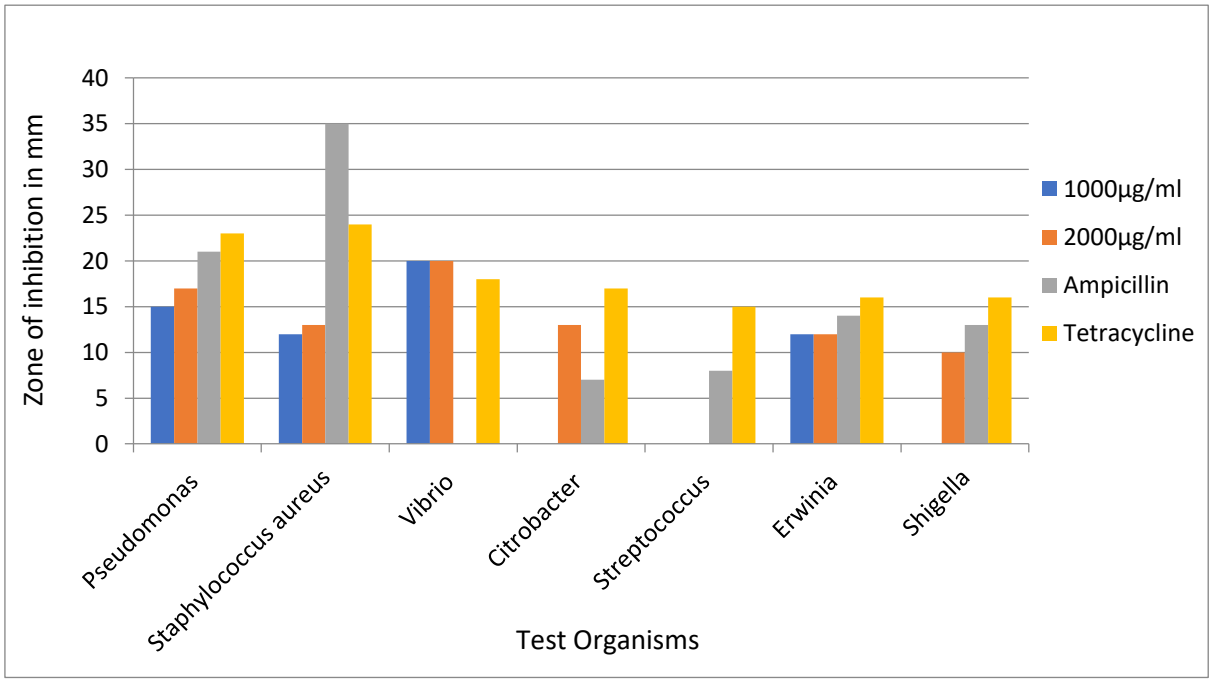

Figure 5: Antimicrobial activity of the extracted pigment from *Epicoccum sorghinum.*

The pigment's capacity for scavenging free radicals was quantified using the DPPH assay, as depicted in **Figure 6**. The concentration required for 50% scavenging of DPPH (IC_{50}) was determined as 95 µg/mL, highlighting the antioxidant activity of the isolated pigment. In a similar investigation conducted by Chen *et al.* (2021), the antioxidant activity of a pigment extracted from *Penicillium expansum*, was evaluated using the DPPH method. The DPPH assay is widely recognized for its ability to measure the radical scavenging capacity of natural extracts. Chen *et al.* (2021) also determined the IC_{50} value of 80 µg/mL, signifying the antioxidant property of the pigment. This closely corresponds with our findings where an IC_{50} value of 95 µg/mL demonstrated strong antioxidant property of the pigment extracted from *E. sorghinum.* These concurrent studies underscore the robust antioxidant potential of pigments derived from diverse fungal sources. This further reinforces their versatility and potential as sources of natural antioxidants with applications in various industries, as well as their potential as contributors to health-promoting products.

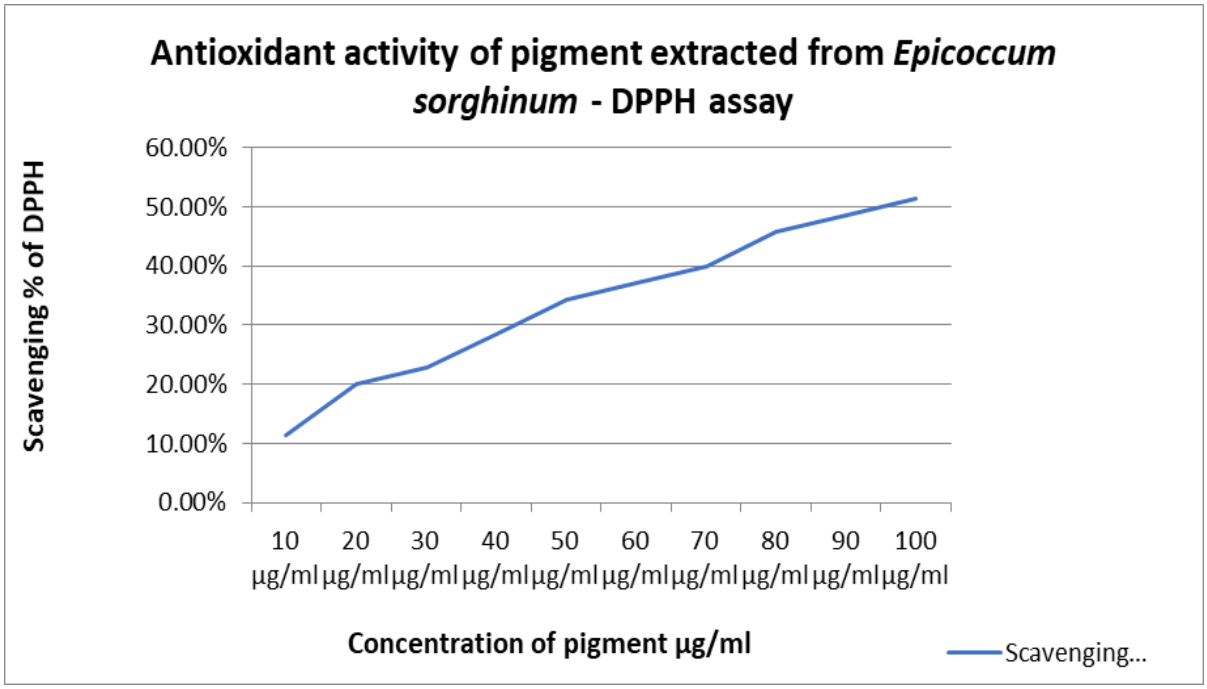

Figure 6: Antioxidant activity of pigment extracted from *Epicoccum sorghinum.*

CONCLUSION

In this research endeavour, we embarked on an exploration of microorganisms from diverse sources in pursuit of pigment-producing entities. Our journey led us to isolate and characterize 3 distinct pigment-producing bacteria and 6 remarkable pigment-producing fungi. Among these fungi, *E. sorghinum* emerged as the focal point of our investigation, mainly due to the limited prior research on its pigment production and potential applications. Our efforts delved into optimizing the conditions conducive to biomass and pigment production, unravelling key insights into *E. sorghinum* behaviour. We discovered that the optimized temperature for pigment production was 25°C, with diminished yields observed at 35°C. Similarly, a neutral pH of 7 was the most favourable environment for pigment production. In the realm of carbon and nitrogen sources, fructose as a carbon source and organic nitrogen sources exhibited their prowess by fostering optimal mycelium growth and pigment production. Our endeavour culminated in the characterization of the crude pigment extract, employing Thin-Layer Chromatography and Gas Chromatography-Mass Spectrometry (GC/MS). The TLC chromatogram unveiled vibrant yellow, orange, and red spots, each representing unique pigment compounds. The subsequent GC-MS analysis meticulously identified and quantified two compounds: Tricyclo[2.2.1.0(2,6)]heptan-3-ol,4,5,5-trimethyl (67.424%) and 11-oxa-dispiro[4.0.4.1]undecan-1-ol (32.576%). Our exploration ventured into the biological realm, where the pigment's effectiveness as an antibacterial agent against Gram negative bacteria and its potent antioxidant activity (IC_{50}95 µg/mL) were revealed. As natural colorants, these pigments have the potential to transcend their aesthetic appeal, offering novel sources of antioxidants and formidable antimicrobial agents. Their applications extend across various industries, including food, textiles, leather dyeing, and pharmaceuticals.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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