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REVIEW ARTICLE

Multifarious pigment producing fungi of Western Ghats and their potential

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Abstract

Concerns about the negative impacts of synthetic colorants on both consumers and the environment have sparked a surge of interest in natural colorants. This has boosted the global demand for natural colorants in the food, cosmetics and textile industries. Pigments and colorants derived from plants and microorganisms are currently the principal sources used by modern industry. When compared to the hazardous effects of synthetic dyes on human health, natural colors are quickly degradable and have no negative consequences. In fact, fungal pigments have multidimensional bioactivity spectra too. Western Ghats, a biodiversity hotspot has a lot of unique ecological niches known to harbor potential endophytic pigment-producing fungi having enumerable industrial and medical applications. Most of the fungi have coevolved with the plants in a geographical niche and hence the endophytic associations can be thought to bring about many mutually beneficial traits. The current review aims to highlight the potential of fungal pigments found in the Western ghats of India depicting various methods of isolation and screening, pigment extraction and uses. There is an urgent need for bioprospecting for the identification and characterization of extremophilic endophytic fungi to meet industry demands and attain sustainability and balance in nature, especially from geographic hotspots like the Western Ghats.

Keywords

Western Ghats, fungi, pigments, endophyte, anticancer, natural colorant

Introduction

Fungi are important components of the environment, with a wide range of structures, functions, and habitats. The ubiquitous nature of the fungi vastly spread in soil, water and air gives a clue of their persistent nature, which in turn is due to many pathways favoring survival mechanisms. They are abundantly found in forests and marine ecosystems. Fungi can be considered to be treasure trove of bioactive compounds, given the fact that they have managed to successfully survive from a very early period in the evolutionary history and have also managed to thrive in diverse habitats under stressed environments. They are primarily involved in organic matter breakdown, biogeochemical cycles, mutualistic interactions and pathogenicity. Based on the plant/fungi ratio, a worldwide estimate of fungi has been derived - between 1.5 and 3 million fungal species. Forest environments of India, the Himalayas and the Western Ghat ranges are key hotspots of fungal diversity (1).

The Western Ghats range is situated in South India covering a total of 160000 km^2 (2). More than 500 plants have been shown to have therapeutic effects. Medicinal plants have long been recognized as a source of fungal endophytes that produce new compounds or help plants in modifying pathways to produce bioactive compounds. Endophytes are those microbes that reside inside the healthy tissues of plants without causing any apparent diseases. Most of the endophytic fungi belong to Ascomycetes. Endophytic fungi have been identified as powerful pigment producers also. Pigments isolated from Western Ghats filamentous fungi include anthraquinone, anthraquinone carboxylic acids, and pre-anthraquinones. Hence, soil from the Western Ghats can be a source of many industrially important fungi (3).

Color has always been important in the lives of all plants and animals on this planet. According to archaeological evidence, natural pigments have been employed as coloring agents from prehistoric times. Due to their applicability in a variety of sectors, synthetic pigments have dominated the market since their discovery. Synthetic colors pose a threat to the environment and human and animal health. The drawbacks of synthetic pigments include poor decomposition, longer persistence and the potential to cause allergies and tumors. Fungal pigments offer several advantages, including easy and rapid growth in a low-cost culture media, varied color shades that are not affected by weather and usage in a variety of industrial applications. All these reasons led to a boost in the demand for organic, natural and eco-friendly pigments in the modern world. Several investigations have shown the use of these fungal pigments in the dyeing of cotton, silk and wool. Changes in agricultural methods, erosion, top soil disruption, mining, ploughing and changes in chemical and physical soil qualities all contribute to a decrease in the variety of endophytic fungal communities in soil. As a result, there is a pressing need to investigate the variety of endophytic fungi in natural settings, with a focus on pigmented fungi, which are under various levels of threat (4). The current review investigates the status of pigment-producing fungal diversity in the Western Ghats area of India and their bioactivities. With a lot of research data on the multifaceted potential of pigmented fungi of the Western ghats available in recent years, it is imperative to probe the utility of the natural products obtained from these fungi in textile and food industry and therapeutics for human health.

Significance of fungal pigments

Natural pigments are those synthesized by microbes, plants and animals. Some of the major pigment-producing plants in widespread use since ancient times include indigo, beetroot, annatto, grapes, turmeric, madder, saffron, etc. Microorganisms are particularly ideal for large-scale pigment manufacturing due to the good comprehension of their cultural procedures, processing and simplicity of handling. Many researchers have reported natural colors from microbes, particularly bacteria and fungi, all over the world. Many bacteria have been shown to have pigment-producing capability, but their pathogenicity and accompanying toxicity have prevented development and commercialization. Due to this, fungal pigments have assumed greater importance for a variety of applications. Cheap culture media, non-seasonality of pigment production, creation of pigments with varied color shades, better stability and solubility of pigments, easy processing, use of waste materials as production substrates etc are some of the benefits of fungal pigments over plant pigments. Melanins, carotenoids, azaphilones, indigo, quinones, phenazines quinones, monascin, indigo and violacein are some of the pigments which fungi produce in a prolific manner (5). The major species of pigment-producing myco-endophytes belong to the genera of *Fusarium*, *Monascus* and *Aspergillus*.

Due to their advantages over harmful synthetic pigments, eco-friendly natural pigments have seen a tremendous demand spike. Colorants, color intensifiers, additives, antioxidants and other chemicals are utilized in a multitude of industries, including textiles, pharmaceuticals, cosmetics, painting, food and beverages. Fungi have recently emerged as one of the most popular and environmentally safe sources of natural colors. They are ideal alternatives to natural pigments due to their ease of processing, rapid growth in low-cost media and weather-independent growth. Thus fungi can be considered as miniature factories making pigments that have prospective applications in a variety of sectors, including pharmaceuticals and textiles.

The first synthetic color developed was mauveine, by Sir William Henry Perkin. Since then, there has been rapid progress in the discovery of different types of synthetic colors. “Coal tar” colors derived from organic compounds like aniline have become quite popular. Due to the ease of production and storage, superior coloring properties, low cost and absence of unintended flavors in the food, synthetic pigments captured the market very quickly. Synthetic dyes found their application in a wide range of fields like textile, pharmaceuticals, cosmetics and paints. Dyes like cochineal red and tartrazine cause allergies when present alone or in combination with other dyes. Some synthetic dyes have been found to promote cancer although they were approved by the US Food and Drug Administration (FDA). Carbon black extensively used as printing ink is carcinogenic and many benzidine dyes cause bowel cancer. The discharge of these dyes into the environment can lead to the accumulation of toxins over a long period of time (6).

Serious allegations were made against these synthetic dyes in a study which showed the relation between artificial colors and hyperactivity in children in Southampton. These dyes included sunset yellow, quinoline yellow, tartrazine, Allura red, carmoisine and ponceau 4R - called “The Southampton Six” (7). Demands for natural pigments have amplified due to these negative effects of synthetic dyes. Apart from the nutritional benefits, natural colorants also give an appetizing look to the food. A good amount of research on the properties and characteristics of these pigments has been discussed. The main challenges faced by the natural color industry include the sensitivity and stability of the pigment and the availability of raw materials. Production of pigments from fungal colonies captured the attention of mycologists in the early 1900s as the fungal kingdom is a natural reserve for food-grade pigments. Fungal pigments are also more advantageous due to the high yield of pigment, highly sustainable nature, cost efficiency, ease of downstream processing and low labor cost (8).

Currently, plants and microbes are used as a primary source for the extraction of colorants by modern industries. In microbial sources, algae and fungi produce a wide range of water-soluble pigments. However, the low yield of pigments from algae is a limitation in exploiting them for pigment production. Filamentous fungi belonging to Ascomycetes or Basidiomycetes are known to produce several pigments like melanins, azaphilones, flavins, quinines etc. Fungi belonging to Basidiomycetes have been used since ancient times in dying silk and wool. Fungi of the genera *Fusarium, Aspergillus* and *Penicillium* produce pigments as intermediate metabolites during their growth (9).

Biodiversity of fungal endophytes in the Western Ghats

The Western Ghats is shared by the six Indian states of Kerala, Tamil Nadu, Karnataka, Maharashtra, Goa, and Gujarat, totaling about 160000 km^2. At various altitudinal ranges (500 to 1200 m above mean sea level), it is home to a diverse range of vegetation including evergreen and deciduous forests, shola woods etc. With 4780 plant species, the Western Ghats is one of the 34 global biodiversity hotspots, with 2180 of these being indigenous to the region. Being widely known for their widespread prevalence and presence of various novel and diverse bioactive compounds, endophytes are microorganisms that colonize plant cells and tissues without causing any apparent injury or disease to their host plants. Among these, fungi are the most commonly encountered types of endophytes (10). Research has proven that these organisms possess numerous types of bioactive compounds and secondary metabolites that have wide-ranging pharmaceutical, industrial and environmental applications. Botanical surveys conducted from 2005 to 2009 revealed that the Western Ghats are home to about 126 aromatic plant species belonging to 61 genera and under 17 families. Nevertheless, bioprospecting of the microbiome associated with this diverse range of plants remains unexplored (11). Research into these organisms dwelling in rare ecological niches has garnered more attention *in lieu* of the novel bioactive compounds present in such endophytic fungi. It was reported that significant antibacterial and antioxidant properties of endophytic fungi isolated from *Nothapodytes foetida*, *Hypericum mysorense* and *Hypericum japonicum* collected from Western Ghats of Karnataka, India. Being the first of its kind to explore the endophytic fungal characteristics of these fungi, this study also depicted the high level of species richness of endophytic fungi present in these plants from Western Ghats (12). Another study dwelled into probing the diversity of fungal assemblages and enzymatic profile of fungal endophytes present in a few endangered plants in the Western Ghats, Karnataka. Among the organisms tested, 29% produced amylase, 28% produced cellulase, 18% produced pectinase, and 40% produced asparaginase (13). In yet another study 3611 fungal isolates were recovered from different herbaceous medicinal plants belonging to the Malnad region, southern Western Ghats. This study revealed the diversity of fungal endophytes in a few plants from this region and their seasonal distribution patterns (14). Other similar studies have also reported wide diversity of species richness and bioprospecting of bioactive compounds produced by fungal endophytes isolated from *Memecylon umbellatum*, an endangered medicinal plant from Western Ghats (15). Revolving around the screening of fungal endophytes from rare or endangered plants, another reported that varieties of mycorrhizal and non-mycorrhizal endophytes from a critically endangered terrestrial orchid *Paphiopedilum druryi* (Bedd.) Stein. This explorative study helped to identify different fungal endophytes namely *Tulasnella calospora*, *Penicillifer martinii* and *Colletotrichum* sp. isolated from root and stem segments of this endangered plant, having colonization rates of 0.27 and 0.23 respectively (16).

The Western Ghat also called Sahyadri is a mega biodiversity zone that starts from the river Tapti in the north to Kanyakumari in the south and runs through the states of Maharashtra, Goa, Karnataka and Kerala, extending over a length of about 1300 km. The Western Ghats covers a wide range of vegetation including deciduous forests, grasslands, evergreen forests, shola forests, scrub jungles and semi-evergreen forests. The forest ecosystem in the Western Ghats and Himalayas harbors about 850 species of macrofungi. Fungi are mainly involved in the decomposition of organic matter by mutualistic associations and biogeochemical cycles. All soil organisms live in a diversified and complicated habitat provided by the rhizosphere. The Western Ghats are regarded as one of the biodiversity hotspots, including microbiological diversity. As a result, soil from the Western Ghats can be a source of industrially important fungi (17).

The Western Ghats, being older than the Himalayas, represents a unique and heritage ecosystem with diverse topography and environmental conditions. High plant species richness found in these regions is thus attributed to the properties of Western Ghats (11, 18). This is regarded as one of the most important biogeographic zones of India, being a tropical evergreen-forested region possessing enormous biodiversity (19). However, only a little work has been done to probe the properties of fungal endophytes in India. Areas like the Western Ghats are hotspots of species richness in terms of plant endemicity and hence these areas can be also attributed to possessing certain novel fungal endophytes that would have evolved along with host plants. Additionally, fungal endophytes residing in rare or endangered plants, rare ecological niches and extreme habitats have high economic value instead of the rich and diverse reserve of novel bioactive compounds. Some of the important pigments from endophytic fungi (20-30) are listed in Table 1 and soil fungi (31-49) in Table 2. The chemical structures of prominent pigments from endophytic and soil fungi are depicted in Supplementary Tables 1 and 2 respectively. Bioprospecting such endophytic fungi would help elucidate various biochemical properties of these organisms.

Table 1. Pigments of prominent endophytic fungi of Western Ghats

Fungal Species	Pigment	Pigment Color	References
Alternaria alternata	Alternariol, Altenuene, Alternarienoic Acid, Tenuazoic Acid, Alterperylenol, Stemphyperylenol	Red Red-violet Red Orange-red Red Yellow-orange-red	(20)
Chaetomium globosum	Chaetoviridins A–D, Chaetoglobin A–B, Chaetomugilins A–F, Cochliodinol	Yellow Red Yellow Purple	(21)
Cladosporium cladosporioides	Calphostins A–D and I	Red	(22)
Curvularia lunata	Chrysophanol, Cynodontin, Helminthosporin, Erythroglaucin, Catenarin	Red Bronze Maroon Red Red	(21) (22)
Fusarium oxysporum	2,7–dimethoxy–6–(acetoxy-ethyl)– Juglone, Bikaverin, Bostrycoidin, Nectriafurone, Norjavanicin, O–methyl–6– Hydroxynorjavanicin, O–methyl-anhydro-fusarubin, O–methyl-fusarubin, O–methyl-javanicin, 2–acetyl–3,8–dihydroxy–6–methoxy anthraquinone, 2–(1–hydroxyethyl)–3, 8–dihydroxy–6–methoxy anthraquinone, Neurosporaxanthin, β–carotene, Uncharacterized naphthoquinones	Yellow Red Red Yellow Red Yellow Orange-red Red Yellow Orange Orange Red-orange Purple	(23) (24) (22) (25) (26)
Hypoxylon sp.	Hypoxyxylerone, Fragiformins A-B, Cytochalasin H, Mitorubrin azaphilones, Vermelhotin, Hypoxylone, Rubiginosin, Hypomiltin	Green Red White Red Orange-red Orange Orange-brown Yellow-green	(21)
Monascus sp.	Citrinin, Monascin, Ankaflavin, Monascorubramine, Rubropunctatin	Yellow-/ Yellow Yellow Red Orange	(22) (27) (21)
	Neurosporaxanthin, Phytoene,	Yellow-orange Yellow-orange	(21) (23)

Neurospora sp.	β–carotene, Neurosporen, Spirilloxanthin, Lycopene	Red-orange Yellow-orange Violet Red	
Ophiocordyceps sp.	Erythrostominone, Deoxyerythrostominol, Epierythrostominol	Red Red Red	(21)
Penicillium sp.	Sorbicillins, Xanthocillin, Chrysogine, Anthraquinones, Citrinin	Yellow Yellow Yellow Yellow Yellow	(22) (27)
Phyllosticta capitalensis	Melanin	Black	(29)
Talaromyces funiculosus	Ankaflavain	Yellow	(22)
Trichoderma sp.	Pachybasin, Chrysophanol, Emodin,	Yellow Orange-red Yellow	(21)
Xylaria polymorpha	Melanin	Black	(30)

Table 2. Pigments of prominent soil fungi of Western Ghats

Fungal Species	Pigment	Pigment Color	References
Aspergillus sp.	Melanin, Aspergillin, Neoaspergillic Acid, Asperversin	Black Black Brown Yellow Yellow	(31) , (28) (32)
Fusarium sp.	Anthraquinone, Naphthoquinone	Pink / violet Yellow	(33) (34)
Monascus sp.	Monascorubrin, Rubropuntatin, Monascorubramine, Rubropuntamine, Ankaflavin, Monascin	Orange Red Red Orange-red Yellow Yellow	(6) (35) (36) (37) (38) (39)
Penicillium sp.	Atronenetin, Anthraquinone, Mitorubrinol, Sclerotiorin, Citromycetin, Citromycin, (−)-2,3-Dihydrocitromycetin	Yellow Red Red Yellow- orange Yellow Yellow	(40) (41) (9) (42) (43) (44) (45)
Trichoderma sp.	Viridol Virone Viridin	Yellow Yellow Yellow- green-brown	(46) (47) (48) (49)

Isolation of fungal endophytes

Pigment-producing fungi can be isolated from soil by serial dilution and plating in an appropriate rich media like Saboraud's Dextrose Agar. It is incubated for 2-3 weeks at 25-28 °C to get fungal colonies. Prominent media used to isolate fungi are Potato Dextrose Agar (PDA), Potato Carrot Agar (PCA) and Czapek Dox Agar (CDA), Melin Norkans Medium (MMN), Malt Extract Media (MEA), Corn Meal Agar (CMA) and Water Agar (WA) (15). Fungal endophytes have an immense potential to produce bioactive compounds. The host plant that harbors the fungi ranges from cryptogams like Thallophyta, Bryophyta and Pteridophyta to phanerogams like gymnosperms and angiosperms (48-50). Different fungi can be extracted from roots, stems, leaves, flowers, fruit, bark and scales. Fungal isolates can also be subcultured from spores or hyphae to get pure cultures. Furthermore, spores that are dried will not germinate, therefore, they must be rehydrated before subculture. Sterile mycelia or the mycelia that fail to sporulate during cultivation must be avoided for this method (51). For inoculation, these fungal spores are mixed with the media at low temperatures. Media is supplemented with an antibiotic like chloramphenicol/streptomycin/oxytetracycline/penicillin/novobiocin to suppress the growth of bacteria.

Screening for fungal enzymes

Screening for amylase production is done by inoculating the isolates in starch agar supplemented with Chloramphenicol and flooding with iodine solution after incubation (52). *Aspergillus niger*, *A. carbonarius* (53), *A. flavus* and *Penicillium notatum* (54) were among the best producers. The genera of *Mucor*, *Rhizopus* and *Fusarium* have also shown high production. Screening for cellulase production is done by inoculating the isolates in cellulose media and flooding them with Congo Red after incubation. *Trichoderma viride*, *Fusarium subglutinans* and *Aspergillus fumigatus* are a few of the prominent species exhibiting this trait. Screening for carboxymethyl cellulase production is done by inoculating the isolates in carboxymethylcellulose media and flooding them with Congo red after incubation. *Aspergillus niger* (54), *A. terreus* (55) and *Trichoderma viride* (56) were found to be some of the best producers. Screening for protease production is done by inoculating the isolates in casein agar and observing for the zone of clearance. *Aspergillus nidulans* (57) and *Fusarium oxysporum* (58) species were found to be good producers of protease. Screening for lipase production is done by inoculating the isolates in mineral media and was observed for opalescence after incubation. Species including *P. notatum*, *Fusarium incarnatum* (59) and *Aspergillus niger* (60) produce lipase.

Extraction of intracellular pigments

For the extraction of intracellular pigments and hydrophobic compounds, green methods of extraction are preferred, as it is organic solvent-free and is, therefore, a safer and more environment-friendly option. Some of these methods take place at a lower temperature, thus owing to better extraction of heat-labile compounds without their degradation. Some of the key methods of pigment extraction are depicted in Fig. 1.

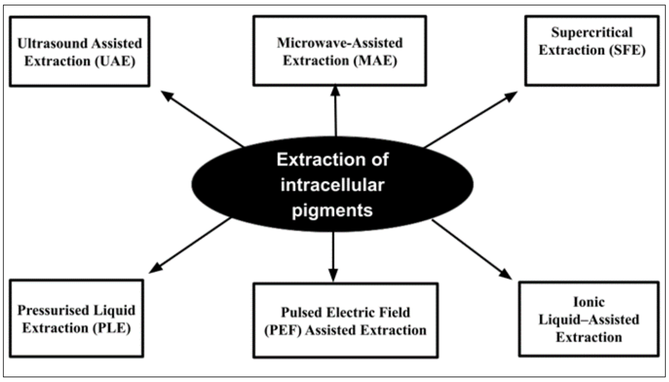

Fig. 1. Methods of extraction of fungal pigments

Ultrasound-Assisted Extraction (UAE)

Ultrasound-Assisted Extraction has been found to be an efficient method for heat-labile pigment production in several phytopharmaceutical industries. UAE results in higher extraction efficiency at lower temperatures. High-intensity ultrasound pressure waves are exploited for fungal pigment extraction from the broth culture. The acoustic energy of the waves is not absorbed by the molecule, but it travels through the broth. These waves generate a local pressure, causing the tissue to rupture and release intracellular substances. The advantage of this technique is that it is simple, and can be done with a smaller quantity of solvent (61-62). UAE has been used in the extraction of pigments from *Boletus edulis*. It is commonly employed in the extraction of pigments and has the potential to improve the stability and extraction yield of bioactive components. The extraction conditions that were used included a liquid-solid ratio of 38 ml/g, an ethanol concentration of 55%, an ultrasound duration of 42 min and an ultrasound power of 450 W, yielding close to 85% yield (63).

Pressurized Liquid Extraction (PFE)

Pressurized Liquid Extraction, also called accelerated solvent extraction has gained popularity due to its reproducibility, low solvent consumption, high extraction yield and low time when compared to Soxhlet extraction, maceration or percolation techniques. This technique makes use of three parameters, simultaneously – high pressure and high temperature. High pressure provides for penetration of the solvent into the sample. At high temperatures the interaction between matrix and analytes is broken, thus increasing the diffusion and solubility of the pigments. In a recent study, the extraction of red pigments from *Talaromyces* by pressurized liquid extraction proved to be much faster and paved a greener way of synthesis when compared to the conventional methods. The advantages of PLE include a significant reduction in solvent usage and low time. Red bikaverin along with two novel purple pigments were extracted from *Fusarium oxysporum* LCP531 using PFE. A six-stage pressurized liquid extraction (PLE) process was used to recover fungal pigments from lyophilized mycelia. This approach allowed multiple solvent polarity profiles to be applied to either lyophilized biomass or fermentation broth, increasing the extraction efficiency of different colors from the same matrix. The use of greater pressure during extraction protects the compounds of interest from oxidation and guarantees that the biochemicals recovered are of better quality (64).

Microwave-Assisted Extraction (MAE)

Microwave-Assisted Extraction uses microwaves of 300MHz to 300 GHz to heat the sample based on dipole moments, a technique tailored for obtaining high-value bioactive compounds and for the production of high-quality extracts. Major factors involved in the efficacy of extraction and separation by MAE include temperature, particle size, pressure, solvent and substrate material. Water is generally the most commonly used solvent for MAE due to its non-toxicity, non-flammability, safety and non-corrosiveness. It falls under the category of green solvents and can be used for the extraction of a wide range of pigments (65). When compared to Soxhlet extraction or maceration, combining MAE with the proper solvent enhanced the amounts of anthraquinones isolated from *Morinda citrifolia* and reduced the extraction time substantially. MAE requires only 15 min to be completed, but Soxhlet and maceration procedures take 4 hrs and 3 days respectively, to achieve the same efficiency (66). As a result, MAE is a very promising approach that has been further refined for reducing side oxidations by employing a nitrogen-controlled environment instead of air, or for protecting heat-sensitive molecules by performing MAE under vacuum conditions. Considerable reduction in extraction time and solvent volume requirements, as well as enhanced extraction efficiency, make MAE an attractive option for biotechnological applications. However, owing to heterogeneous heat propagation at larger sizes, as well as the material's cost and maintenance, its industrial applications are restricted (67).

Supercritical Extraction (SFE)

Supercritical extraction is a novel method of extraction using liquified carbon dioxide gas as the supercritical fluid (68). This technique works on the principle of solvating the power of gasses above their critical limit. Non-polar compounds are mainly extracted using supercritical CO_2 since they are hydrophobic in nature. However, it can be made polar by using a co-solvent like ethanol for the extraction of hydrophilic pigments. The efficacy of extraction can be increased by manipulating the pressure, flow rate, amount of co-solvent and temperature of these gasses (68-69). Furthermore, as the physicochemical characteristics of water can be changed with temperature and pressure, its uses are becoming even more diverse, such as in subcritical extraction methods for less polar substances. Carbon dioxide is also extensively used in extraction procedures under supercritical conditions. Carbon dioxide has become popular for widespread use as a supercritical fluid due to its favorable supercritical temperature and pressure characteristics (31 °C and 73.8 bar), as well as its affordability and environmental friendliness (70). This method has been mostly used in the extraction of carotenoids (71).

Ionic Liquid Assisted Extraction

Ionic liquids (IL) are solvents that have the potential to lead to the creation of new extraction technologies that are gentler, greener and more efficient. Ionic liquids are solvents that are made for high efficacy extraction and purification of bioactive compounds. Some of the special qualities of IL that designate them as green solvents are low vapor pressure and non-volatile nature, non-flammability, thermal and chemical stability, high solubility and recycling ability. It has a wide range of salt combinations; therefore, it can overcome the limited selectivity of substrates. Ionic liquids have the added advantages of being more economical and reducing environmental footprint. A combination of water or organic solvents can be used with ILs to increase efficiency (72).

More recently, researchers have focused on combining IL with other techniques such as the aqueous two-phase system (ATPS), microwave-aided extraction and ultrasound-assisted extraction to boost the IL's extraction capabilities. Similar procedures combining IL with ultrasound, microemulsion or ATPS were used with satisfactory results on filamentous fungal biomass and/or culture broth. Anthraquinones from *Penicillium purpurogenum* have been successfully extracted using this method (72). There was similar success extracting red *Monascus* pigments from the 7-day-old fermentative broth using hydrophobic IL microemulsions (73).

Pulsed Electric Field (PFE) Assisted Extraction

Pulsed Electric Field (PFE) Assisted Extraction is a non-thermal extraction method that works on the principle of electropermeabilization. Here, the cell is exposed to high-intensity electric waves which cause the breakdown of the cell membrane, thus assisting the release of intracellular pigments. Increased permeation of the solvents into the cell is also a factor that aids in the release of these pigments. Process parameters of PFE include duration, frequency and intensity of electric waves, which can be manipulated for the release of various pigments and bioactive components from the cell. A study has discussed the combination of PFE and solvent extractions at different pH for the extraction of carotenoids and other bioactive components, by using a lower quantity of organic solvents (74).

Fermentation and extraction of extracellular pigments

For easy downstream processing and extraction of water-soluble pigments, a submerged type of fermentation is preferred. Generally, two major types of submerged fermentation are used for the production of pigments. The first type is the fed-batch system where fresh media is added slowly and continuously in accordance with a targeted feeding regime (75, 76). The next type of feeding is the continuous system, wherein the exponential phase is delayed by the continuous inflow of fresh media and removal of spent media (77). For fungal fermentation, a suspension of spores or vegetative cells has been used as inoculum. The inoculum of spores is however preferred over vegetative cells, owing to its advantages like high viability, ease of handling, maintenance, stability and preservation (78). Media optimization is an important parameter for the growth of fungi for maximum biomass production to enable a high yield of pigments. It involves modification of culture environment such as altering carbon or nitrogen source, changing culture parameters like pH, temperature, aeration, light intensity etc. Change in the pH of the culture medium affects the concentration of pigments (79). In a study, it was concluded that a minimal medium like minimal dextrose broth favored low pigment and high biomass production (66). Additionally, complex forms of nitrogen and carbon in potato dextrose broth along with cofactors like magnesium, iron, zinc and copper enables higher pigment production (64).

Production of citric acid from *A. niger* is done by submerged fermentation. It was found that sugar, protons and oxygen when found in excess, nitrogen and phosphate when found in limiting conditions and trace elements and manganese when found below defined limits were optimum for the production of citric acid. Apart from these, pH, temperature and aeration had a significant effect on citric acid production (80) It was reported that maximum biomass production for *Isaria fumosorosea* was achieved after 36 hrs of submerged fermentation with media containing nitrogen and carbon organic sources (81). Yellow pigments produced by *Thermomyces* sp. in submerged fermentation using sucrose and ammonium sulfate as carbon and nitrogen sources shows that an increase in pH, temperature, or light intensity does not affect the stability of the pigments and was moderately stable in antioxidants and preservatives (82). With a view to offset manufacturing costs, researchers have shown keen interest in optimally utilizing industrial by-products or waste materials for fermentation operations for the production of microbial pigments (83). Low-cost substrates have been favored for the production of fungal pigments. In place of yeast extract corn steep liquor has been used successfully as a nitrogen source for the production of pigment from *Monascus ruber* (84).

Applications of fungal pigments

The plethora of pigments from fungi has a multitude of functions encompassing environmental stress protection (melanins), serving as cofactors in enzyme catalysis (flavins) and protection against deadly photo-oxidations (carotenoids) to protect against environmental stress (melanins) and functioning as cofactors in enzyme catalysis (flavins). Natural colorants are produced by a range of fungal species found in soil niches and have several industrial applications. In the food sector, these colorants are employed as additives, color intensifiers and antioxidants. In the textile industry, they are used as textile dyes. Furthermore, anthraquinones are employed in the production of antimicrobial textiles. Currently, the importance and utilization of these pigments is rocketing. It would be difficult to identify an industry where these pigments do not play an important role. The food sector faces a significant difficulty in finding colors that are both ecologically friendly and capable of long-term use. When pigments are in the form of dispersions, mass coloring can be performed in textile fibers, polymers and rubber.

Filamentous fungi have been shown to generate a wide variety of pigments such as carotenoids, melanins, flavins, phenazines, quinones, monascins, violacein and indigo. Despite several obstacles, fungal pigments have made it to the market and are now competing with synthetic pigments. Various food-grade fungal pigments from *Ashbya gossypii, Monascus*, *Penicillium oxalicum*, *Blakeslea trispora* etc are currently found in global markets (85). More than 200 fungal species have been found to produce carotenes (39). Order Mucorales (Class: Zygomycota) which has the following genera - *Blakeslea*, *Mucor* and *Phycomyces* - was found to be abundant in Carotenes (86).

Textile Colorants

The textile industry makes use of more than 13 lakh tonnes of synthetic dyes and dye precursors (87). Most of these dyes survive normal wastewater treatment processes and remain in the environment due to their high instability to light, chemicals, detergents, heat, soap, water and other characteristics such as perspiration and bleach. Furthermore, several of these colors include potential colon carcinogens, which represent a risk to people when exposed to them on a long-term basis (88). As a result, there is an increasing need for non-hazardous and eco-friendly colorants, especially for food coloring and textile dyeing. Several researchers have reported the use of these natural dyes and pigments in the dyeing of cotton, silk and wool samples (89). Fungal pigments are environmentally friendly colorants. Anthraquinone from *Fusarium oxysporum* can be used to dye wool fabrics (22). *Penicillium minioluteum* pigment was used to color wet blue goat Nappa skin (90). Fungal pigments are known to be stable at various temperatures. Dyes for pre-tanning leather samples from *Monascus purpureus, Fusarium* spp. and *Penicillium* spp. have been found to be stable at high temperatures (91).

Purified pigments of *A. niger* were used to color fabrics such as cotton, silk and silk cotton, which were extracted from soil samples and inoculated in potato dextrose broth. UV spectrophotometer was used to evaluate the absorption percentage of purified dyes onto the fabrics. Textiles that had been pre-mordanted had a higher percentage of dye absorption than fabrics that had not been pre-mordanted. Cotton fabrics were found to be more effectively taking up the dye when compared to silk and silk cotton fabrics. Because the pigments were employed to color wool, the dye generated had a high reflectance at 482 nm (7). *F. oxysporum* anthraquinone chemicals have been utilized as natural dyes to color wool. Purified pigments from *P. purpurogenum* pose an excellent option for the natural coloring of cotton materials and have a lot of potential for producing antibacterial activity and antibacterial finishes, which are employed in medical textiles (92). *P. oxalicum* NRC M25 yielded a light reddish-brown pigment used in textile dyeing (7).

The biocatalysts in the fungal pigments increase the swelling of leather fibers and thereby accelerate the pigment diffusion into the leather samples. Improved fungal pigment exhaustion will not only minimize pollution but will also result in human-safe leather goods. *Isaria* spp., *Emericella* spp., *Fusarium* spp. and *Penicillium* spp. have been used to dye leathers since they have the ability to produce pink, red, yellow and reddish-brown colors. These pigments were proved to be non-toxic and are obviously biodegradable. Temperatures around 70 °C have proven optimum due to the high activity of the functional molecules. The leather quality was found unaffected by fungal pigments. Organoleptic characteristics were found to be equivalent to those of chemically dyed leather. The enzymes generated by filamentous fungi have been claimed to be used in the leather dyeing process to make the softer leather. As a result, the presence of these enzymes in pigments causes the fibrous leather network to open up, allowing colors to penetrate more easily. Furthermore, pigment enzymes can demonstrate some binding, which can accelerate the exposure of a greater number of functional sites for pigment binding. The resultant fungal pigment may be spray-dried or lyophilized to produce a variety of powder colorants and the solvent ethanol can be recovered using an appropriate solvent recovery technique. When compared to hazardous synthetic dyes, the environmental element and health problems involved in the dyeing application outweigh the cost factor (91). Without the use of mordants, a unique yellow pigment derived from *Aspergillus* sp. MBYP1 was shown to be effective for cotton and silk (93).

Antimicrobial activity

The growth of multidrug-resistant bacteria throughout the world and the lack of enough drugs to tackle such diseases remains a serious worry for the medical community globally. Many pigmented fungi with antimicrobial activity have been found in Western Ghats. *Monascus ruber* pigment showed antibacterial action against food-borne microorganisms (94). Human pathogenic bacteria such as *Vibrio cholerae, Klebsiella pneumoniae* and *Staphylococcus aureus* were inhibited by the pigment of an endophytic fungus, *Monodictys castaneae* (95). Strain MF5 of *Penicillium* sp. collected from the Western Ghats which generated green pigment exhibited broad-spectrum efficacy, MF2 of *Aspergillus* sp. which generated black pigment inhibited *Bacillus subtilis* and *E. coli*. Among other strains, MF5 demonstrated broad-spectrum activity, hence it was chosen as a viable strain for further research (15). When compared to the antibiotic ciprofloxacin, the red pigment generated by *M. purpureus* demonstrated good antibacterial activity and was shown to be 81% effective (96). Pencolide and sclerotiorin from *P. sclerotiorum* were found to have antibacterial action against Gram-positive bacteria such as *Staphylococcus aureus*, *Salmonella typhimurium* and *Streptococcus pyogenes* as well as Gram-negative bacteria like *Candida albicans* and *E. coli.* Isochromophilone was found to have antibacterial properties against *S. aureus* (97). *Aspergillus sclertiorum* DPUA 585 generated neoaspergillic acid, a yellow pigment with antibacterial and antifungal properties against *E. coli, Staphylococcus aureus* and *Mycobacterium smegmatis* (26). *Penicillium* species have been found to have antibacterial action against *Bacillus cereus, Listeria monocytogenes* and *Candida albicans* (98). *P. purpurogenum* produced more antibacterial extracellular pigments in the dark, a trait that could be beneficial in the industries. *P. purpurogenum* developed increased extracellular pigments with antibacterial activity in darkness, which might be used in the pharmaceutical and healthcare industries (99). *P. purpurogenum* produced more antibacterial extracellular pigments in the dark, which might be useful in the healthcare and pharmaceutical industries.

Food Colorants

Due to the detrimental health effects linked with many synthetic colors, food companies have recently shifted from synthetic to natural colors. Furthermore, the food sector has significant problems in developing healthy antimicrobial and antioxidant products. Carotenoids and betanins, for example, are pigments used in the food industry that contain labile hydrogen that readily undergo oxidative decolorization as they are light, heat and oxygen-sensitive. These characteristics restrict the color additives' resilience during the preparation, storage and presentation of foods to which they have been added (100). To make food items more attractive, many colors from fungal sources are currently being explored, including the beautiful red pigments from *Monascus* sp., astaxanthin from *Xanthophyllomyces dendrorhous*, Arpink redTM from *Penicillium oxalicum*, riboflavin from *Ashbya gossypii*, β-carotene from *Blakeslea trispora* and lycopene from *Fusarium* sp. (101).

One of the safety concerns while using fungal pigments in food is the issue of toxic molecules - for example, citrinin of *Monascus*. Much of the research on *Monascus* pigments are being focused on techniques to reduce citrinin synthesis or establish strains that are unable to co-produce citrinin (102). *P. oxalicum* produces red pigment of the anthraquinone class, which has beneficial qualities such as water solubility and a wide range of colors. They also do not require the addition of stabilizing agents to foods (103). The oldest documented use of fungal pigment in food was for the creation of red-mold rice (ang-kak). Oriental cuisine (in Southeast Asia, China, and Japan) makes extensive use of such *Monascus* pigments including the yellow (ankaflavine, monascine), orange (rubropunctatine, monascorubrine) and purple (rubropunctamine, monascorubramine) (39).

Carotenoids are utilized commercially as animal feed supplements and food colorants. They find a way in treating obesity too. Carotenoids are commercially employed as food colorants and animal feed additives and are also used to treat obesity. They have been employed in nutraceutical, cosmetic and medicinal applications (104). Canthaxanthin is a pigment found in foods such as cheese, candies, fish, meat, fruits, drinks, snacks, beer and wine. Riboflavin (vitamin B2) pigments are used in drinks, quick desserts and ice creams. Carotenoids can function as sunscreen to protect food from ultraviolet rays, therefore preserving its quality (101). Pigments extracted from *Monascus* had 92-98% stability when added to sausages at 4 ^{0}C for three months (105). Although various fungal pigments have been described in the literature, they must comply with specific requirements concerning toxicity, regulatory approval, stability and the financial expenditure necessary to scale up production (106, 107). To avoid toxin production, non-pathogenic strains are best chosen wisely to control toxin production (39).

Antioxidant Activity

With an increase in free radicals in the body, the risk of chronic illnesses such as autoimmune disorders, diabetes and cancer rises by way of neutralizing the free radicals (108). Anthraquinones from the endophytic fungus *Stemphylium lycopersici* have shown antioxidant activity (109). The antioxidant activity of the microbial pigments is mostly due to the polyene chain and the functional OH/NH groups which are capable of scavenging free radicals (93). In textile industries, to improve the light fastness, dyed fabrics are usually coated with antioxidants (110). Another research examined the efficacy of coated cotton fabrics with vitamin E in cosmetotextile applications as a skin-protection and anti-aging product (111). The yellow pigment of *Aspergillus* sp., isolated from soil showed the maximum antioxidant activity, which was higher than that of the standards such as ascorbic acid and BHT. Since the fungal pigments naturally possess antioxidant properties, they have a potential in textile industries reducing the need for the use of coated antioxidants in fabrics (93). Another endophytic fungus, *Talaromyces purpureogenus* KKP was isolated and characterized from the soil in Keekan village, Kasaragod district, which had good antioxidant activity and lower IC_{50} value, indicating good potential as a food colorant (112).

The orange pigment (1,2-dimethoxy-3H-phenoxazin-3-one), isolated from the fungus *Gonatophragmium triuniae* which infects the leaves of the *Maytenus rothiana*, a plant endemic to central Maharashtra (Western Ghats) has been shown to possess anti-tumoral, antioxidant, antiproliferative and antibacterial properties. The hexane extract of this pigment showed promising antioxidant properties (113). A yellow-colored pigment isolated from the *Penicillium sclerotiorum* strain AK-1 showed significant antioxidant capacity (114). Reactive oxygen species cause stress and have a negative impact on cells, causing them to shut down their regular machinery. The inability of antioxidants to counteract the effects of reactive oxygen species (ROS) is a major factor in the onset of age-related diseases. Antioxidants control and limit the cellular and molecular damages caused by ROS. Natural pigments have been proven to have antioxidant properties. The pigment extracted from *Penicillium mallochi* ARA1 has shown good antioxidant potential (115).

Anticancer activity

Many studies have been made to test fungal pigments for successful anticancer treatment. Anthraquinone derived from *Alternaria* sp. an endophytic fungus has been tested on breast cancer cells (116). Pigments derived from *Monascus* spp. have outstanding anticancer efficacy against several types of cancers. *Monascus* pigments, such as monascin, inhibited mouse skin carcinogenesis. Ankaflavin inhibited HepG2 and A549 human cancer cell lines. HEp-2 human laryngeal carcinoma cell lines have been inhibited by monaphilone A and monaphilone B (117). *Monascus* metabolites have been found to have prophylactic and therapeutic potential, based on clinical and preclinical research. Many fatty acids, especially butyric acid and pyran were found in abundance in a *Monascus ruber* strain AUMC 5705. All these compounds have been proven to possess anticancer effects (34). This revelation has led to the creation of novel anticancer *Monascus*-based pharmaceutical and food items to alleviate human malignancies (35). *Penicillium* sp. generates several secondary metabolites with high bioactive chemicals, which are utilized in pharmacy to make medications to treat a variety of ailments, as well as in agriculture. Endophytic fungi are expected to harbour more industrially important enzymes when obtained from stressed environments (118).

The fungus *G. triuniae* produces orange pigment which is identified as 1,2-dimethoxy-3- *H*-phenoxazin-3-one which possesses anticancer, antitumor and antiproliferative activity (113). Among the metallic NPs, copper and its oxide forms exhibit anti-cancer, anti-insect, antioxidant, antibacterial, quorum quenching, catalytic, antiviral and anti-helminthic properties (119). Endophytes that live with the host plant/tree are mutualistic and endophytic fungi produce metabolites that are identical to those produced by the host plant/tree. As a result, metabolites found in both intracellular and extracellular mycelia would eventually cause the NP substrate to produce the appropriate NPs (120). The anti-cancer potential of CuNPs and CuONPs from an indigenous species of *Trichoderma* had been effectively assessed using a photothermal treatment for human lung carcinoma (121). CuONPs produced by the endophytic fungus *A. terreus* FCBY1 had the highest biological activity, including antibacterial, antioxidant and anti-cancer properties. On HT- 29 cell lines (colon cancer cell lines), CuONPs exhibited the highest scavenging mechanism and showed good anti-cancer action (122). Some of the prominent fungi from Western ghats and the pigments they produce are depicted in Supplementary Fig 1.

Conclusion

The present societal preference for organic ingredients, as well as public concern about the negative effects of synthetic pigments on the environment and human health, has reignited the demand for natural colorants. In recent decades, there has been a steady increase in the use of diverse biotechnological instruments to offer nutritious, appealing, healthy and high sensory quality goods, making this procedure more cost-effective and ideal for mass applications. Despite the fact that nature provides a bountiful supply of safe colors, fundamental constraints such as raw material availability and pigment profile variation associated with colors derived from plants drive the color industry toward the promise of colors derived from microbial sources, particularly fungal resources. Apart from their use as colorants in food and textile industries, fungal pigments find increasing uses as antibacterial, antioxidant and anticancer elements. In addition, the fungal pigments are natural and do not have any negative impacts when released into the environment. The tremendous fungal variety of the Western Ghats is yet to be fully explored for identifying useful novel bioactive compounds.

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Authors contributions

SS conceived the study and participated in its design. Data collection and manuscript drafts were done by BS, NRR, BPP and SJ. Reviewing and editing was done by SS. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest:

Ethical issues: None.

Supplementary data

Supplementary Table 1. Chemical structures of pigments of prominent endophytic fungi of Western Ghats

Supplementary Table 2. Chemical structure of pigments of prominent soil fungi of Western Ghats

Supplementary Fig. 1. Prominent pigments of fungi in the Western Ghats

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