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Article of Studies on melanin production by some filamentous fungi

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ABSTRACT *: Melanin pigments are found in a wide variety of creatures in nature, including human, microorgasmis and plants.Potential uses for human health exist in the breakdown of melanin. For instance, by degrading melanin to make tumours sensitive to radiation therapy, it might improve the treatment of melanoma. The oxidation of tyrosine produces indoles and other intermediate intermediates, which are present in the irregular light-absorbing polymer known as melanin, Melanin granules, the primary pigment of hair and epidermis, are transported from melanocytes to epithelial cells. There are numerous biological uses for melanin. Melanins are biological macromolecules that are dark, typically black, and made up of different phenolic or indolic monomers. They are frequently complex with proteins and carbohydrates. Animals, plants, protozoans, and microorganisms all make melanin. These studies highlighted the urgent need for a thorough Paper on the melanin pigment isolated from microorganisms. This paper covering biosynthesis, bioproduction, characterization, and potential applications would aid researchers from various backgrounds in comprehending the significance of microbial melanins and in using the information for planning melanin-related studies. With this objective in mind, the current research contrasts traditional and cutting-edge concepts for environmentally sustainable melanin extraction techniques.*

KEYWORDS: *Bioremediation, Antioxidant, Characterization, Microbial melanin, Anti-cancer activity*

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I. INTRODUCTION

Very dark and black brown pigment, complex hydrophilic irregular polymer is the pigment melanin. It is created in a variety of species through the oxidative polymerization of phenolic or indolic chemicals **[1].** Melanins are tolerant to oxidizing substances, free-radicals, radiation and heat. Melanin, in general, can shield bacteria from ionizing radiation **[2].** By absorbing electromagnetic spectrum and protecting living creatures from visual damage, melanin has anti-UV radiation properties. As demonstrated, melanin can produce itself in response to anti-fungal medications **[3].** Melanin granules are not soluble in water and popular organic solvents (such as he-xane, chloro-form, ethanol, me-thanol, or acetone) **[4].** Due to their detrimental effects, which may be attributed to their complicated chemical structures when compared to natural ones, consumers are now worried about the chemical synthetic components of such pigments. It is believed that in these factories, the production of microbiological cells is straightforward and quick [5]. Natural pigments derived from microbial sources are used in the food, cosmetics, and industrial sectors without harming the environment or contaminating goods **[6].** According to d'Ischia et al., the melanins are a family of polymeric pigments that are abundantly present in nature **[7].** These are the byproducts of phenolic or indolic substrates being oxidised by enzymes. Melanins are regarded to be one of nature's oldest pigments. These hues can be seen in the remains of dinosaurs and birds [8]. Remarkably, intact melanins were discovered in Jurassic-era squid ink sacs **[9].** So, the study of evolution is proposed to use melanin as a biomarker **[10].** According to D'Ischia et al., melanins perform a wide range of tasks, including photoprotection, the removal of reactive oxygen species, the sequestration of hazardous metals, thermoregulation, and camouflage **[7].** Melanins can be classified as brown to black eu-melanin and yellow to reddish pheo-melanin **[11,12].** These melanin subgroups differ not just in terms of hue but also in terms of their photochemical and other characteristics **[13,14]**. The fact that the majority of naturally occurring melanin pigments are co-polymers of eumelanin and pheomelanin is another crucial component of melanin formation **[15].**

Eu-melanin, pheo-melanin, allo-melanins, and pyo-melanin are the four primary forms of melanin.The amino acids L-tyrosine and/or dihydroxyphenylalanine are oxidised to produce eu-melanin (L-DOPA). Afterward, the polymer takes on a brown or black hue.L-tyrosine and L-DOPA are oxidised in the presence of L-cysteine to create pheomelanin, a pigment with a reddish-yellow. The oxidation of hydroxy-phenylacetic acid, cat-echols, di-hydroxynaphthalene (DHN), -glutaminyl-4-hydroxy-benzene, or tetrahydroxy-naphthalene, protocatec-hualdehyde, and caffeic acid produces the allo-melanin. One kind of melanin produced by the oxidation of homogentisic acid is called pyo-melanin. (Figure 1), **[16]**

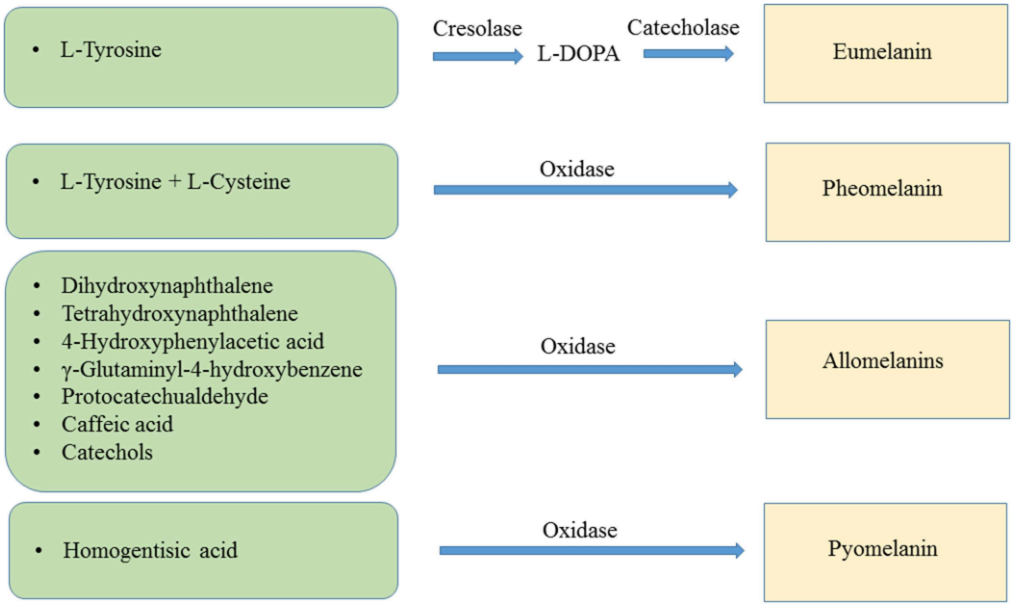

Fig (1): The biochemical processes that produce eu-melanin, pheo-melanin, allo-melanins, and pyo-melanin.

1-ENZYMES DIRECTLY CONNECTED TO MELANIN

The initial process leading to the production of melanin is the enzyme-dependent ox-idation of phenolic or indole chemicals. Tyrosinase and laccase protein families make up the majority of the enzymes that cause melanogenesis. The most prevalent class of enzymes connected to melanogenesis are tyrosinases. Both mono- and diphenolic substances are acceptable substrates for these enzymes.These substrates include catechols, L-tyrosine, and L-DOPA, as examples.The mono-oxygenases known as tyrosinases have a copper catalytic core with two nuclei.These enzymes catalyse the oxidation of catechols and the ortho-hydroxylation of monophenols, producing ortho-quinone compounds Fig 1**[17].**

Tyrosinase enzyme uses molecular oxygen-mediated oxygenation processes to catalyze the hydroxylation of L-tyrosine to L-DOPA. It subsequently oxidizes this chemical to dopachrome, which non-enzymatically polymerizes to produce melanin **[18].** Based on their amino acid sequences and functional properties, microbial tyrosinases can be divided into five primary types **[19].**

2-Melanin biological functions

Additionally, these polymers display redox activity and can scavenge re-active oxygen species and free_ radicals [20]. Melanin is being investigated as a component of electrical circuits, batteries, and solar cells since it is an amorphous semiconductor [21]. These applications are now being used with inorganic semi-conductors, they are cheap, yet they have a big influence on the environment. Organic semi-conductors, like melanin, do not have these issues and are simpler to work with. Melanin has extra benefits over conventional semi-conductors, including the ability to be used in implantable devices thanks to its bio-compatibility. In a different kind of application, melanin has been used as a way to create nanostructures and nanoparticles made of silver or gold that could be useful in the food and medical industries **[22].** Melanin has also been suggested as a synthetic polymer ingredient. Thermal stability of poly (methyl -methacrylate) (PMMA) was discovered

to significantly enhance with the addition of eu-melanin **[23].** Similar research has shown that allo-melanin can be added as adye to the hydrogel of soft contact lenses **[24].**

The utilization of melanin has the advantage of having antibacterial, antioxidant, and antifungal activity when compared to synthetic colours. Melanin is used in cosmetic products, including hair dye. The common synthetic oxidative dyes harm hair and are difficult to apply. Contrarily, a method based on the usage of melanin precursors that can shield hair from oxidation after exposure to air has the benefit of not harming it and being safe **[25].**

Applications in the environment can make use of melanin's capacity to act as metal chelators, the binding of metals to melanin requires several coordination bonds between the hydroxyl, amine, and carboxyl functional groups in this polymer. Melanin from fungi has been demonstrated in a soil bioremediation study to effectively bind heavy metals, including zinc and lead **[26].** Tyrosinase from the plant Amorphophallus campanulate was used to produce melanin in a later investigation using l-DOPA as a substrate. It was found that uranium may be effectively removed from an aqueous solution by melanin **[27].**

Although it is uncertain which chemical dyes have been substituted with pigment in this case, melanin's natural origin and ability to block out strong visible light are recognized as benefits. At the moment, melanin is mostly used commercially as a dye for sunglasses lenses. Acommercial sun-screen for arid skin that contains squid ink as an antioxidant is relevant to dermatology. When compared to synthetic dyes, this product is projected to cause reduced skin sensitivity, which gives it an advantage over competing sunscreens **[5].**

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Fig 2: Functional properties of melanin **[28]**

II-Factors affecting on production of melanin:

As a result of its potential for use in a variety of industrial processes, including the manufacture of foods, cosmetics, pharmaceuticals, and textiles, microbial pigment synthesis is currently one of the most promising areas of research. However, it is well recognised that selecting the right productive culture strain and figuring out the ideal growing conditions are essential for the success of microbial fermentation operations **[29].**

An ideal microbe for making pigments should be able to use a variety of carbon and nitrogen sources, be tolerant of PH, temperature, mineral concentrations, and produce a decent number of pigments. Additionally, recommended characteristics are simple separation from cell biomass and nontoxic and nonpathogenic natures. Because filamentous fungi may be grown under a variety of environments, including oligotrophic or nutrient-rich settings, a wide range of temperatures (10-50 ^{o}C), pH (2 to11), salinity (0 to 34%), and water activity (0.6 to 1), they have a great deal of metabolic plasticity. It is crucial to determine the nutritional and en-vironmental elements that have a higher impact on the growth of cell and production of pigments produced by microbial fermentation in order to increase efficiency & reduced costs **[30].**

According to certain research, the sufficient aeration is necessary for pigment synthesis. This is possibly because various enzymatic processes that produce pigment depend on oxygen, for cells to function properly, carbon and nitrogen are required, and these sources are related to biomass formation,

the type of pigment generated, and the yield of the desired chemical. Nutrients may control the expression of specific genes and activate vital metabolic pathways involved in pigment synthesis **[31].**

Generally speaking, glucose, an essential carbon source for growth, inhibits the synthesis of numerous secondary metabolites, including pigments. Studies have shown that a nitrogen source's ability to promote or inhibit the synthesis of pigment depends on the strain. It has been demonstrated that different kinds of peptone and yeast employed as a nitrogen source can promote an increase in the production of pigments in a wide variety of fungus species **[32].**

Medium composition change is a key tactic to increase pigment synthesis since some sources of carbon and nitrogen can support higher yields of the desired product and can be more easily digested. In order to make the bioprocess economically viable on an industrial scale, it is necessary to choose inexpensive and effective substrates for the manufacture of pigment since they have an impact on the cost of the bioprocess. Strong industry interest in using large quantities of agro-industrial waste produced by various economic activities as cheap materials to support the development of microorganisms in bioprocesses has been sparked by the high amounts of these wastes **[33]**. Melanin may have increased significance for the sector and aids in reducing or preventing pollution-related waste from entering the environment **[34].**

III -Pathways of melanin biosynthesis

Different methods have been used to clarify the structure of melanin from various organisms, such as electron paramagnetic resonance **[35],** X-ray diffraction **[36],** infrared, ultraviolet, and visible spectroscopy **[37],** and nuclear magnetic resonance **[38].** These investigations have demonstrated that by oxidatively polymerizing phenolic or indolic chemicals, fungi are able to create several forms of melanin **[39**]. When O-di-phenolic substances, such as 3,4-dihydroxy-phenylalanine, are present in the growth media, Apathogenic Basidiomycetous is known to produce DOPA-melanin. To increase its capacity to manufacture melanin, this fungus may employ a variety of substrates, including L-dopamine **[40],** catecholamines, and other phenolic chemicals **[41].** This fungus polymerizes exogenous substrates as a result of laccase activity **[42].** It is crucial to stress that melanin generated from various substrates exhibits a variety of features.

Figure 3 is a general diagram of the manufacture of fungi's dihydroxy-naphthalene (DHN)-melanin is shown in figure 3. Malonyl-CoA is transformed into 1,3,6,8-tetra-hydroxynaphthalene (1,3,6,8-THN) in this pathway by the poly-ketide synthase (PKS), which then undergoes a number of reduction and dehydration events to create scytalone, 1,3,8-tri-hydroxynaphthalene (THN), and vermelone. The intermediate 1, 8-dihydroxy-naphthalene (DHN), which is produced by a subsequent dehydration step, is p0lymerized into DHN_melanin, probably by a laccase enzyme**[43].**

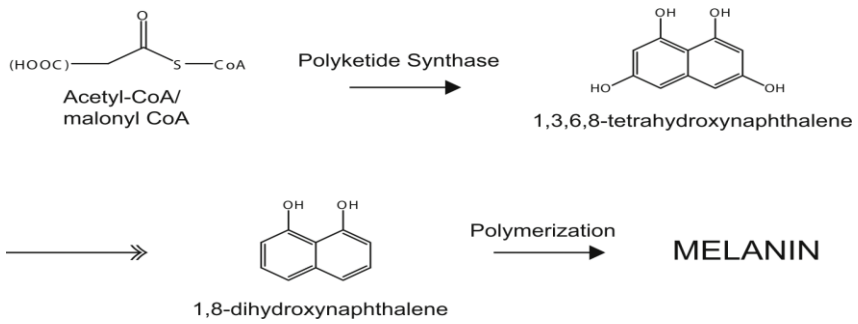

Figure 3: The metabolic pathway of fungal di hydroxy naphthalene (DHN)-melanin, which depicts a broad model for fungal dihydroxy-naphthalene (DHN)-melanin bi0synthesis **[43].**

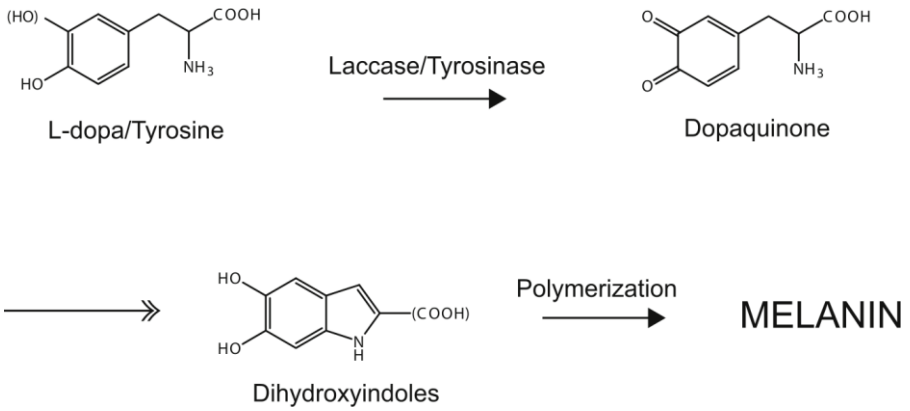

Figure 4. Dihydroxy-phenylalanine (DOPA)-melanin's synthesis process in fungus **[43]**

L-dopa and tyrosine are two potential beginning chemicals in this pathway. If L-dopa is the precursor molecule, laccase will then oxidize it to dopaquinone. Tyrosine is transformed into L-dopa and subsequently dopaquinone if it serves as the precursor. Both processes are carried out by the same enzyme, tyrosinase. Leucodopachrome is created by the extremely reactive intermediate dopaquinone, which is then oxidized to produce dopachrome. Dihydroxy indoles are produced by hydroxylation (and decarboxylation), and they can spontaneously polymerize to generate DOPA-melanin **[38].**

Some fungi have multiple melanin biosynthetic pathways. For instance, Aspergillus fumigatus produces DHN-melanin by the tyr0sine degradation pathway, as well as a second type of melanin that protects the hyphal cell wall from ROS. Gray-green DHN-melanins also control the structural integrity of the cell wall of conidia and their important properties **[44].** Moreover, melanin is important for plant pathogen host invasion. Appressoria are structures that fungi develop that penetrate plant tissue and allow the organisms to invade the host. These structures' the cell walls contain melanin, which gives them mechanical strength and promotes tissue penetration **[45].** Melanized appressoria have been shown to have a great importance in the pathogenicity of certain plant infections, such as the rice blast fungus and the rose black spot disease **[46].**

IV- Descriptions of melanin

Melanin's diverse makeup prevents it from having a distinctive, well-defined structure. As a result, several techniques are needed to establish the structure of melanin, including a stringent set characterization procedure. and to confirmed by (chemical and physical method, UV- visible, Fourier-transform infra-red spectro-scopy, NMR spectro-scopy, High performance liquid chromatography **[47, 12].**

a – Ultra.violet–visible spectro-scopy

Numerous studies shown that although just a little amount of ultra- violet rays (280-315 nm) reach the earth's surface, they have a significantly unfavorable effect on living things. It causes the pyrimidine adduct, which inhibits transcription and translation. By absorbing energy, melanin will block these approaching UV rays **[48].** Maximum UV spectrum absorption for microbial melanin occurs between 200 and 400 nm.UV light absorption is caused by the presence of complex conjugated molecule structures in melanin **[49].**

Elsayis confirmed that the UV-visible absorption spectrum pattern was obtained by both the extracted melanin from different micr-oorganism and synthetic melanin, revealing that the absorption peak was at the Ultra-Violet region and declined towards the visible region with high points of absorption at 240 nm and 219 nm respectively **[50].**

b- Physico-chemical properties

As microbial melanin exhibits differential solubility, the most frequent stage for the initial identification and characterization of melanin is solubility. Di-methyl sulphoxide (DMSO), alkaline water (PH more than 8), and phosphate buffer saline (pH 7.2) show solubility in melanin, which makes them the most suitable solvents for extraction steps **[51]**. Melanin is mostly insoluble in water and various some important solvents, but it also shows solubility in these solutions. The breakdown of melanin caused the pigment to become discolored when it was exposed to hydrogen peroxide (H2O2). The mechanism of the reaction also involves the nucleophilic assault of O-OH ions from H2O2, which triggers a ring-opening reaction that

produces the quinone e-poxides that bleach melanin **[52].** Grey precipitation is produced on the test tube walls as a result of the reaction between microbial melanin and $AgN0_3$ solution **[53].**

Melanin precipitates as a result of the reduction of AgN03**[54].** As for a different method of detection, the presence of KMn04 results in the pigment's color changing from brown to green, precipitation, and then the solution being dis-coloured. Melanin undergoes a reduction of KMn04 because of the presence of electron donor groups, which is what led to the change in hue.These reactions demonstrate that the melanin component contains quinolinic and phenolic groups**[55].**

c- Fourier-transform infra-red spectro-scopy

IR spectrum of *Hortaea werneckii* derived pigment showed a number of significant peaks near 3438 cm-1, 2927 cm-1, 1637cm-1, and 1239.90 cm-1. The initial peak indicated the presence of the (-OH) group, and the band's enlargement may be explained by the amino group's (-NH) hydrogen bonding with the (-OH) group (Barretto and Vootla.,2020). According to Dullah et al., showed FTIR in other studies that Peaks were seen in the FT-IR spectra at 3399 cm-1, 2926 cm-1, 2851 cm-1, 1,586 cm-1, 1385 cm-1, 1030 cm-1, and 618 cm-1. The polymeric O-H groups are responsible for the absorption at 3399 cm-1. At 2926 cm-1 and 2851 cm-1, the stretching vibrations for aliphatic C-H bonding may be observed. The symmetric carboxylate stretching vibrations (COO-) are audible at 1586 cm1. At 1385 cm1, the in-dole ring's shaking and CNC stretching were noticed. At 1030 cm 1, CH in-plane/CH out-of-plane deformation is ascribed. The aromatic carbon-hydrogen bond's out-of-plane bending is indicated by the value of 618 cm1. These functional groups are indicators of melanin content **[47].**

d - NMR spectroscopy

Since determining the 1H NMR spectrum of microbial melanin is a key step in determining the structure of the pigment, several researchers have turned to this cutting-edge methodology. Chemical shifts are provided in ppm downfield from SiMe4, with the residual solvent signal serving as an internal reference, Bayram demonstrated. signals between 6 and 8 ppm are associated with the aromatic groups, according to the overall pattern (indole units) (Bayram et al., 2020). Microbial melanin's ^{1}H NMR spectra included peaks linked to the aliphatic region of alkyl fragments as well as peaks of $-CH_2$ or $-CH_3$ groups linked to nitr0gen or 0xygen atoms. Peaks in the aromatic region may be related to the indole rings found in the structure of melanin at 7.2–8.0 ppm, according to the aliphatic region **[56].**

e- High performance liquid chromatography

The HPLC analysis was carried out using the Sun et al. Previous research has shown that melanin pigment produced by L-tyrosine has a different peak time than melanin produced spontaneously by environmental microorganisms **[6,57].**

V-Applications for melanin

a- Bioremediation for heavy metals:

Melanin had a high affinity and binding ability for a number of heavy metals, including Cu^{+2}, Zn^{2+} Mg^{+2}, Cd^{2+}, and Mn^{2+} **[58]**. A functional group of pigments interacts with this metal-binding characteristic. Utilizing melanin to recover and find valuable heavy metals from solutions or to bioremediate polluted water is made possible by the interaction of melanin with metal **[59,60]**. Nguyen et al confirmed that the effective removal of Cr^{6+} and Mn^{2+} by melanin was over 97%, and the adsorption capacities for Cr^{6+} and Mn^{+2}were 5.78 mg/gam and 31.8 mg/gam, respectively. More intriguingly, it showed that melanin could remove vibrio parahaemolyticus bacteria with an efficiency of more than 90%, in addition to heavy metals. The obtained results suggested that melanin, a naturally occurring substance with a high level of biosafety, would be useful adsorbent for removing bacteria and heavy metal ions in aqueous solution and might be employed for beneficial water treatment **[61].**

b- Nano-technology

The creation of more stable, monodispersible, environmentally benign metallic nanoparticle synthesis is a key aspect of modern nanotechnology **[62].** Metal nanoparticle production uses the biopolymers made by microorganisms as a reducing and stabilising agent **[63].** It is an investigated biopolymer because it has intriguing qualities like chelating metal ions, photosensitization, photoprotection, and antibacterial Therefore, bacterial or fungal melanin is in competition with the creation of nanoparticles and their use in a variety of industries, including food packaging, paint additives, and cosmetics **[64].**

c- textile sector:

Melanin is a polymeric component that is very secure, biodegradable, and possible natural colours and can also successfully replace synthetic dyes, despite the textile industry being harmful to the environment. *Streptomyces virginiae*, a species of bacteria, produces dark brown melanin, which is extracted and used for a variety of purposes including dyeing and printing wool materials, which displayed strong coloration **[65].**Lasio-diplodia, aobroma used to colour bleach-ed poplar veneers, secretes melanin, demonstrating its suitability as a dyeing material **[66].**Allo-melanin, which was recently reported to have been obtained from the actinomycete Streptomyces glaucescens, was used to dye cotton fabric, and the intensity of the colour was easily boosted by laccase enzyme treatment **[67].**

d- Anti cancer activity:

The purified melanin pigment plays a very important role in controlling cancer and getting rid of cancer cells, some scientific showed that in other reports **[68].**

A promising anticancer effect of black melanin on HEPG-2 and HCT-116 cell lines, with IC50 values that were higher than those of doxorubicin (4.05 and 4.45 g) in both casesExtra-cellular melanin produced by the fungus *Schizophyllum* commune was shown by Arun et al. to be effective against the human epidermoid larynx carcinoma cell line (HEP-2) in a concentration-dependent manner, indicating its potential use in chemotherapy and cancer chemoprevention **[69].**

Also, MTT assay results in previous studies showed that BTCZ31 melanin inhibited the development of the L929 cell line at a cytotoxic concentration of 105.4 g/mL (IC50). On aberrant cells, morphological modifications included being smaller, rounder, and with angles **[70].**

e- Anti viral activity: -

Melanin can stop pathogens from entering the skin and mucous membranes, and soluble melanin can stop HIV replication. Melanin may lower the prevalence of HIV infection through sexually acquired skin lesions, lowering the probability of sero-conversion and delaying the onset of AIDS **[71].** They proposed that black people's melanin levels may be related to their resistance to HIV infection. The human immunodeficiency virus (HIV) is the root cause of acquired immune deficiency syndrome (AIDS) (AIDS) **[72].**

VI- CONCLUSIONS AND PERSPECTIVE:

Due to its numerous biological features and ability to be environmentally sustainable, melanin supports a variety of uses. Because they may be easily scaled up while preserving a wide structural variety, microorganisms have become a valuable source of melanin. The dedicated biosynthetic pathways that are mostly influenced by enzymatic imbalances are where these sophisticated biopolymers' intricacy is found. Precursor metabolites play a crucial part in this process since their fluctuating concentrations change the metabolic pathways. The antimicrobial activity of melanin is very an important side to explore. Upscaling the bioproduction of melanin is greatly aided by identification of important regulators in biosynthetic pathways& medium optimization in fermentation. Because of its numerous physicochemical properties, melanin is a group of naturally occurring, affordable, abundant, and harmless pigment compounds with many uses. It is simple to extract melanin from a variety of natural sources or to chemically create it. Studies into the structure and function of melanin are still necessary in order to utilize melanin-based materials, despite the fact that melanin and melanin-like compounds are rather well known. Melanin is the ideal pigment for photoprotection and UV radiation protection. Some conditions and environmental factors can decrease or increase the amount of melanin in the skin. Due to its potential cytotoxic action against a number of cancer cell lines and low cytotoxicity against healthy noncancerous cells, the melanin pigment has been proposed as a new natural anticancer agent. There are still a number of issues with all the applications highlighted in the review that need to be resolved. Although it has yet to realise its full potential, melanin extracted from micro-rganisms proves a bright future, primarily in dermatol0gy, biomedicine, cosmetic, agri-cultural, and environmental technologies.

Melanin has a great future in research, especially as it relates to improving bioproduction and extraction methods to accomplish tremendous biotechnological objectives. The family of natural compounds known as melanin can be thought of as functional polymers with numerous potential industrial and medical uses.

V. REFERENCES

[1] Patil, S., Sistla, S., Bapat, V., & Jadhav, J. (2018). Melanin-mediated synthesis of silver nanoparticles and their affinity towards tyrosinase. Applied biochemistry and microbiology, 54(2), 163-172.

[2] Cordero, R. J., Vij, R., & Casadevall, A. (2017). Microbial melanins for radioprotection and bioremediation. Microbial Biotechnology, 10(5), 1186.

[3] Kurian, N., & Bhat, S. G. (2014). Bacterial melanins. *Microbial Bioproducts*, *1*, 97-110

[4]Varga, M., Berkesi, O., Darula, Z., May, N. V., & Palágyi, A. (2016). Structural characterization of allomelanin from black oat. *Phytochemistry*, *130*, 313-320.

[5] Martínez, L. M., Martinez, A., & Gosset, G. (2019). Production of melanins with recombinant microorganisms. Frontiers in bioengineering and biotechnology, 7, 285.

[6] Sun, S., Zhang, X., Sun, S., Zhang, L., Shan, S., & Zhu, H. (2016). Production of natural melanin by Auricularia auricula and study on its molecular structure. Food Chemistry, 190, 801-807.

[7] d'Ischia, M., Wakamatsu, K., Cicoira, F., Di Mauro, E., Garcia-Borron, J. C., Commo, S., ... & Ito, S. (2015). Melanins and melanogenesis: from pigment cells to human health and technological applications. Pigment cell & melanoma research, 28(5), 520-544.

[8] Zhang, F., Kearns, S. L., Orr, P. J., Benton, M. J., Zhou, Z., Johnson, D., et al. (2010). Fossilized melanosomes and the colour of Cretaceous dinosaurs and birds. Nature 463, 1075–1078. doi: 10.1038/nature0874.

[9] Glass, K., Ito, S., Wilby, P. R., Sota, T., Nakamura, A., Bowers, C. R., ... & Simon, J. D. (2012). Direct chemical evidence for eumelanin pigment from the Jurassic period. Proceedings of the National Academy of Sciences, 109(26), 10218-10223.

[10] Westerhof, W. (2006). The discovery of the human melanocyte. Pigment Cell Res.19, 183–193. doi: 10.1111/j.1600-0749.2006. 00313.x

[11] Ito, S., & Wakamatsu, K. (2008). Chemistry of mixed melanogenesis—pivotal roles of dopaquinone. Photochemistry and photobiology, 84(3), 582-592.

[12] d'Ischia, M., Wakamatsu, K., Napolitano, A., Briganti, S., Garcia-Borron, J. C., Kovacs, D., ... & Ito, S. (2013). Melanins and melanogenesis: methods, standards, protocols. Pigment cell & melanoma research, 26(5), 616-633.

[13] Szewczyk, G., Zadlo, A., Sarna, M., Ito, S., Wakamatsu, K., & Sarna, T. (2016). Aerobic photoreactivity of synthetic eumelanins and pheomelanins: generation of singlet oxygen and superoxide anion. Pigment cell & melanoma research, 29(6), 669-678.

[14] Ito, S., Wakamatsu, K., & Sarna, T. (2018). Photodegradation of eumelanin and pheomelanin and its pathophysiological implications. Photochemistry and photobiology, 94(3), 409-420.

[15] Del Bino, S., Ito, S., Sok, J., Nakanishi, Y., Bastien, P., Wakamatsu, K., & Bernerd, F. (2015). Chemical analysis of constitutive pigmentation of human epidermis reveals constant eumelanin to pheomelanin ratio. Pigment cell & melanoma research, 28(6), 707-717.

[16] Lindgren, J., Moyer, A., Schweitzer, M. H., Sjövall, P., Uvdal, P., Nilsson, D. E., & Kear, B. P. (2015). Interpreting melanin-based coloration through deep time: a critical review. Proceedings of the Royal Society B: Biological Sciences, 282(1813), 20150614.

[17] Garcia-Molina, F., Muñoz, J. L., Varón, R., Rodriguez-Lopez, J. N., García-Cánovas, F., & Tudela, J. (2007). A review on spectrophotometric methods for measuring the monophenolase and diphenolase activities of tyrosinase. Journal of Agricultural and Food Chemistry, 55(24), 9739-9749.

[18] Ito, S. (2003). A chemist's view of melanogenesis. *Pigment cell research*, *16*(3), 230-236.

[19] Fairhead, M., & Thöny-Meyer, L. (2012). Bacterial tyrosinases: old enzymes with new relevance to biotechnology. New biotechnology, 29(2), 183-191.

[20] Liu, Y. C., Chen, S. M., Liu, J. H., Hsu, H. W., Lin, H. Y., & Chen, S. Y. (2015). Mechanical and photo-fragmentation processes for nanonization of melanin to improve its efficacy in protecting cells from reactive oxygen species stress. Journal of Applied Physics, 117(6), 064701.

[21] Ambrico, M., Vecchia, N. F. D., Ambrico, P. F., Cardone, A., Cicco, S. R., Ligonzo, T., & d'Ischia, M. (2014). A Photoresponsive red-hair-inspired polydopamine-based copolymer for hybrid photocapacitive sensors. Advanced Functional Materials, 24(45), 7161-7172.

[22] Patil, S., Sistla, S., Bapat, V., & Jadhav, J. (2018). Melanin-mediated synthesis of silver nanoparticles and their affinity towards tyrosinase. Applied biochemistry and microbiology, 54(2), 163-172.

[23] Shanmuganathan, K., Cho, J. H., Iyer, P., Baranowitz, S., & Ellison, C. J. (2011). Thermooxidative stabilization of polymers using natural and synthetic melanins. Macromolecules, 44(24), 9499-9507.

[24] Ahn, S. Y., Choi, M., Jeong, D. W., Park, S., Park, H., Jang, K. S., & Choi, K. Y. (2019). Synthesis and chemical composition analysis of protocatechualdehyde-based novel melanin dye by 15T FT-ICR: High dyeing performance on soft contact lens. Dyes and Pigments, 160, 546-554.

[25] Koike, K., & Ebato, A. (2013). One-pack hair dye compositions containing indole compounds. Jpn Tokkyo Koho, 2013.

[26] Forgaty, R. V., & Tobin, J. M. (1996). Fungal melanins and their interactions with metal. Enz Microb Technol, 19, 311-317.

[27] Saini, A. S., & Melo, J. S. (2013). Biosorption of uranium by melanin: kinetic, equilibrium and thermodynamic studies. Bioresource technology, 149, 155-162.

[28] Roy, S., & Rhim, J. W. (2021). New insight into melanin for food packaging and biotechnology applications. Critical Reviews in Food Science and Nutrition, 1-27.

[29] Kumar, C. G., Mongolla, P., & Pombala, S. (2018). Lasiosan, a new exopolysaccharide from Lasiodiplodia sp. strain B2 (MTCC 6000): structural characterization and biological evaluation. Process Biochemistry, 72, 162-169.

[30] Akilandeswari, P., & Pradeep, B. (2016). Exploration of industrially important pigments from soil fungi. Applied microbiology and biotechnology, 100(4), 1631-1643.

[31] Zou, Y., & Tian, M. (2017). Fermentative production of melanin by Auricularia auricula. Journal of Food Processing and Preservation, 41(3), e12909.

[33] Pombeiro-Sponchiado, S. R., Sousa, G. S., Andrade, J. C., Lisboa, H. F., & Gonçalves, R. C. (2017). Production of melanin pigment by fungi and its biotechnological applications. Melanin, 47-75.

[32] dos Reis Celestino, J., de Carvalho, L. E., da Paz Lima, M., Lima, A. M., Ogusku, M. M., & de Souza, J. V. B. (2014). Bioprospecting of Amazon soil fungi with the potential for pigment production. Process Biochemistry, 49(4), 569-575.

[34] Lopes, F. C., Tichota, D. M., Pereira, J. Q., Segalin, J., de Oliveira Rios, A., & Brandelli, A. (2013). Pigment production by filamentous fungi on agro-industrial byproducts: an eco-friendly alternative. Applied biochemistry and biotechnology, 171(3), 616-625.

[35] Enochs, W. S., Nilges, M. J., & Swartz, H. M. (1993). A standardized test for the identification and characterization of melanins using electron paramagnetic resonance (EPR) spectroscopy. Pigment cell research, 6(2), 91-99.

[36] Crippa, R., Horak, V., Prota, G., Svoronos, P., & Wolfram, L. (1990). Chemistry of melanins. In The alkaloids: chemistry and pharmacology (Vol. 36, pp. 253-323). Academic press.

[37] Wilczok, T., Bilińska, B., Buszman, E., & Kopera, M. (1984). Spectroscopic studies of chemically modified synthetic melanins. *Archives of biochemistry and biophysics*, *231*(2), 257-262.

[38] Duff, G. A., Roberts, J. E., & Foster, N. (1988). Analysis of the structure of synthetic and natural melanins by solid-phase NMR. Biochemistry, 27(18), 7112-7116.

[39] Langfelder, K. M., Streibel, B., Jahn, G., & Haase, A. A. Brakhage (2003).Melanins.Annu. Rev. Phytopath, 24, 411-451

[40] Eisenman, H. C., Mues, M., Weber, S. E., Frases, S., Chaskes, S., Gerfen, G., & Casadevall, A. (2007). Cryptococcus neoformans laccase catalyses melanin synthesis from both D-and L-DOPA. Microbiology, 153(12), 3954-3962.

[41] Garcia-Rivera, J., Eisenman, H. C., Nosanchuk, J. D., Aisen, P., Zaragoza, O., Moadel, T., ... & Casadevall, A. (2005). Comparative analysis of Cryptococcus neoformans acid-resistant particles generated from pigmented cells grown in different laccase substrates. *Fungal Genetics and Biology*, *42*(12), 989-998.

[42] Gessler NN, Egorova AS, Belozerskaya TA. Melanin pigments of fungi under extreme environmental conditions (Review) (2014). Applied Biochemistry and Microbiology.;50(2):105–113.

[43] Gómez, B. L., & Nosanchuk, J. D. (2003). Melanin and fungi. *Current opinion in infectious diseases*, 16(2), 91-96.

[44] Schmaler-Ripcke, J., Sugareva, V., Gebhardt, P., Winkler, R., Kniemeyer, O., Heinekamp, T., & Brakhage, A. A. (2009). Production of pyomelanin, a second type of melanin, via the tyrosine degradation pathway in Aspergillus fumigatus. Applied and environmental microbiology, 75(2), 493-503.

[45] Chen, Z., Nunes, M. A., Silva, M. C., & Rodrigues Jr, C. J. (2004). Appressorium turgor pressure of Colletotrichum kahawae might have a role in coffee cuticle penetration. Mycologia, 96(6), 1199-1208.

[46] Gachomoz, E. W., Seufferheld, M. J., & Kotchoni, S. O. (2010). Melanization of appressoria is critical for the pathogenicity of Diplocarpon rosae. Molecular Biology Reports, 37(7), 3583-3591.

[47] Dullah, S., Hazarika, D. J., Goswami, G., Borgohain, T., Ghosh, A., Barooah, M., ... & Boro, R. C. (2021). Melanin production and laccase mediated oxidative stress alleviation during fungal-fungal interaction among basidiomycete fungi. IMA fungus, 12(1), 1-17.

[48] Gao, Q., & Garcia-Pichel, F. (2011). Microbial ultraviolet sunscreens. Nature Reviews Microbiology, 9(11), 791-802.

[49] Pralea, I. E., Moldovan, R. C., Petrache, A. M., Ilieș, M., Hegheș, S. C., Ielciu, I., & Iuga, C. A. (2019). From extraction to advanced analytical methods: The challenges of melanin analysis. International journal of molecular sciences, 20(16), 3943.

[50] Elsayis, A., Hassan, S. W., Ghanem, K. M., & Khairy, H. (2022). Optimization of melanin pigment production from the halotolerant black yeast Hortaea werneckii AS1 isolated from solar salter in Alexandria. BMC microbiology, 22(1), 1-16.

[51] K**amarudheen, N., Naushad, T., Rao, K.V.B., 2019**. Biosynthesis, characterization and antagonistic applications of extracellular melanin pigment from marine Nocardiopsis sps. Indian J. Pharma. Edu. Res. 53, s112–s120. https://doi.org/10.5530/ijper.53.2s.55.

[52] Korytowski, W., & Sarna, T. (1990). Bleaching of melanin pigments. Role of copper ions and hydrogen peroxide in autooxidation and photooxidation of synthetic dopa-melanin. *Journal of Biological Chemistry*, *265*(21), 12410-12416.

[53] Lopusiewicz, Ł. (2018). Scleroderma citrinum melanin: isolation, purification, spectroscopic studies with characterization of antioxidant, antibacterial and light barrier properties. World Scientific News, 94(2), 114-129.

[54] Carriel, V.S., Aneiros-Fernandez, J., Arias-Santiago, S., Garzon, I.J., Alaminos, M.,Campos, A., 2011. A novel histochemical method for a simultaneous staining of melanin and collagen fibers. J. Histochem. Cytochem. 59, 270–277. https://doi.org/ 10.1369/0022155410398001.

[55] Aghajanyan, A.E., Hambardzumyan, A.A., Hovsepyan, A.S., Asaturian, R.A.,Vardanyan, A.A., Saghiyan, A.A., 2005. Isolation, purification and physicochemical characterization of water-soluble Bacillus thuringiensis melanin. Pigment Cell Res. 18, 130–135. https://doi.org/10.1111/j.1600-0749.2005.00211.x

[56] Amin, S., Rastogi, R.P., Sonani, R.R., Ray, A., Sharma, R., Madamwar, D., 2018. Bioproduction and characterization of extracellular melanin-like pigment from industrially polluted metagenomic library equipped Escherichia coli. Sci. Total Environ. 635, 323–332. https://doi.org/10.1016/j.scitotenv.2018.04.107.

[57] Eskandari, S., & Etemadifar, Z. (2021). Biocompatibility and radioprotection by newly characterized melanin pigment and its production from Dietzia schimae NM3 in optimized whey medium by response surface methodology. Annals of Microbiology, 71(1), 1-13.

[58] Hong, L., Liu, Y., & Simon, J. D. (2004). Binding of Metal Ions to Melanin and Their Effects on the Aerobic Reactivity. Photochemistry and Photobiology, 80(3), 477-481.

[59] Sono, K., Lye, D., Moore, C. A., Boyd, W. C., Gorlin, T. A., & Belitsky, J. M. (2012). Melanin-based coatings as lead-binding agents. Bioinorganic Chemistry and Applications, 2012.

[60] Thaira, H., Raval, K., Manirethan, V., & Balakrishnan, R. M. (2019). Melanin nano-pigments for heavy metal remediation from water. *Separation Science and Technology*, *54*(2), 265-274.

[61] Nguyen, T. L. N., Pham, T. H., & Nguyen, D. T. (2016). Natural melanin as a potential biomaterial for elimination of heavy metals and bacteria from aqueous solution. VNU Journal of Science: Natural Sciences and Technology, 32(1S).

[62] Banerjee, K., & Ravishankar Rai, V. (2018). A review on mycosynthesis, mechanism, and characterization of silver and gold nanoparticles. BioNanoScience, 8(1), 17-31.

[63] El-Batal, A. I., & Al Tamie, M. S. (2016). Optimization of melanin production by Aspergillus oryzae and incorporation into silver nanoparticles. Der Pharm Lett, 8, 315-33.

[64] Ju, K. Y., Lee, Y., Lee, S., Park, S. B., & Lee, J. K. (2011). Bioinspired polymerization of dopamine to generate melanin-like nanoparticles having an excellent free-radical-scavenging property. Biomacromolecules, 12(3), 625-632.

[65] Amal, A. M., Abeer, K. A., Samia, H. M., Nadia, A. E. N. H., KA, A., & HM, E. H. (2011). Selection of Pigment (Melanin) production in Streptomyces and their application in Printing and Dyeing of Wool Fabrics. Research Journal of Chemical Sciences _ISSN, 2231, 606X.

[66] Liu, Y., Zhang, Y., Yu, Z., Qi, C., Tang, R., Zhao, B., ... & Han, Y. (2020). Microbial dyes: dyeing of poplar veneer with melanin secreted by Lasiodiplodia theobromae isolated from wood. Applied microbiology and biotechnology, 104(8), 3367-3377.

[67] Ahn, S. Y., Jang, S., Sudheer, P. D., & Choi, K. Y. (2021). Microbial production of melanin pigments from caffeic acid and L-tyrosine using Streptomyces glaucescens and FCS-ECH-expressing Escherichia coli. International journal of molecular sciences, 22(5), 2413.

[68] Gamal Shalaby, A. S., Ragab, T. I. M., Helal, M. M. I., & Esawy, M. A. (2019). Optimization of Bacillus licheniformis MAL tyrosinase: in vitro anticancer activity for brown and black eumelanin. Heliyon 5: e01657

[69] Arun, G., Eyini, M., & Gunasekaran, P. (2015). Characterization and biological activities of extracellular melanin produced by Schizophyllum commune (Fries).

[70] Kurian, N. K., Nair, H. P., & Bhat, S. G. (2015). Evaluation of anti-inflammatory property of melanin from marine Bacillus spp. BTCZ31. Evaluation, 8(3).

[71] Manning, J. T., Bundred, P. E., & Henzi, P. (2003). Melanin and HIV in sub-Saharan Africa. Journal of Theoretical Biology, 223(1), 131-133.

[72] Kaminsky, L. S., McHugh, T., Stites, D., Volberding, P., Henle, G., Henle, W., & Levy, J. A. (1985). High prevalence of antibodies to acquired immune deficiency syndrome (AIDS)-associated retrovirus (ARV) in AIDS and related conditions but not in other disease states. Proceedings of the National Academy of Sciences, 82(16), 5535-5539.