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Melanin: progress, prospects, and challenges in synthesis and commercial applications

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ABSTRACT

Melanin is a naturally occurring polymer found in a wide variety of organisms, actively involved in biological processes to withstand adverse environmental conditions. Its scientific activities that include UV protection, antioxidant properties, dehydration capabilities, and relatively inexpensive separation of natural systems have made it an optional combination of spectrum systems. Its desirable properties including biocompatibility, biodegradability, metal chelation, and electronic conductance have expanded its use, such as its use in organic semiconductors, drug delivery, environmental remediation, and the cosmetics industry. This article focuses on comparing the natural sources of melanin, its biochemistry, isolation methods, and applications downstream. The use of natural melanin for commercial purposes by existing research spaces and challenges is also included in this article.

Keywords: Melanin, antioxidant property, biocompatibility, environmental remediation and biodegradation.

1. Introduction

The word "Melanin" is derived from the ancient Greek word "Melanos" which means Black as the pigment color appears black usually. The term melanin was first used by the swedish chemist Berzelius in late 1840s. Melanin production is observed in all taxa from both Prokaryotes and Eukaryotes and have been explained in several ways from past 50 years due to its huge diversity in colour, composition and function. A copper-containing monooxygenase, Tyrosinase catalyses melanin synthesis in melanocytes (ElObeid, Kamal-Eldin, Abdelhalim, & Haseeb, 2017). Melanin is an easy-to-find natural biopolymer that can be found among organisms that exhibit various biological processes and helps to protect living organisms from adverse environmental factors. It is used in organic semiconductors as well as, drug delivery, natural bioremediation, and cosmetics. It is a strong antioxidant and has a limited and expensive source of natural melanin and surpasses all aspects of its biocompatibility and biodegradability, as well as metallic properties, metal chelation, and electronic conductance. Melanin metal chelators are very helpful in natural functioning. According to the cosmetics industry melanin has been shown to be used in sunburn and dyeing hair (Tran-Ly, Reyes, Schwarze, & Ribera, 2020). Melanin is a natural dye that can be considered an active ingredient for a variety of uses in the industry. The current review deals with the various sources of melanin and its biochemical structure, also highlight the nature and method of melanin production. Microbial bio-synthesis of melanin is also discussed, which includes the mechanism and method of melanin formation and the use of melanin in various fields with gaps and challenges (Bloomfield & Alexander, 1967).

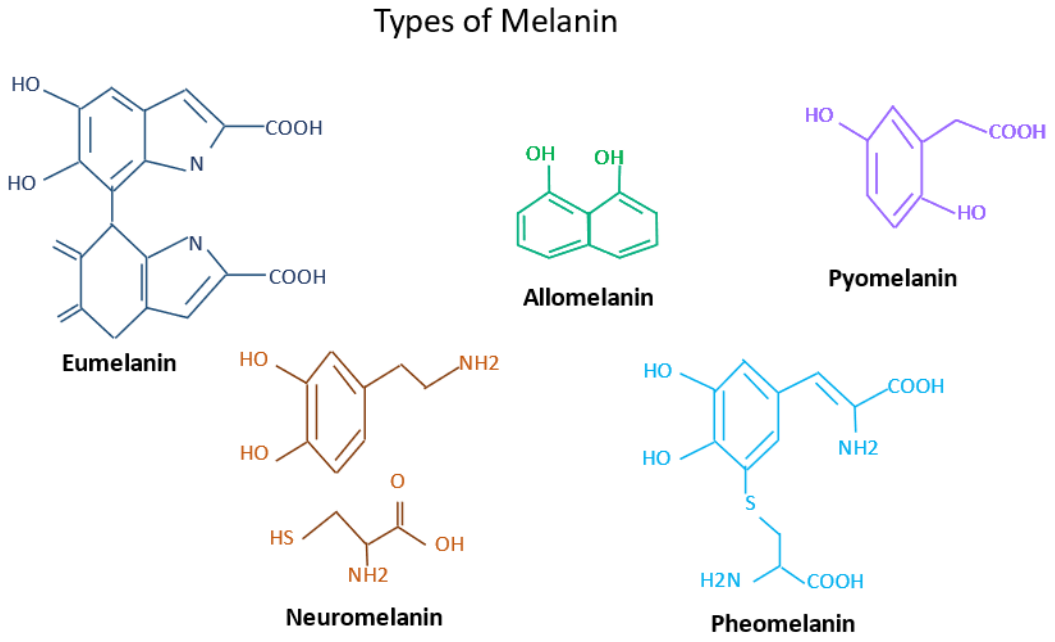

Figure 1 showing Biochemical structure of different types of Melanin

1.1 Nature of melanin

Melanin is a cryptic pigment produced by all domains of life organisms from bacteria to mammals. Melanin has unique feature to absorb radiations and serve as an antioxidant. Among humans, melanin is a pigment responsible for the color of skin and eyes secreted by melanocyte cells in the epidermal basal layer (Bull, 1970). It catalyses the hydroxylation of tyrosine and *o*-diphenol product, *l*-dopa oxidation. This oxidation of DOPA produced an intermediate which is further oxidized to form melanin. Melanin is a biopolymer that is insoluble in aqueous or organic solvents, they are amorphous in nature. Some chemical treatments can able to dissolve melanin such as a strong base but usually changes its real structure or the initial polymer may break into fragments. Melanin is a substance that is dark in colour, resistant to a concentrated acid, and susceptible to bleaching by oxidizing agents (Butler & Day, 1998). Melanin is composed of polymerized phenolic or indolic compounds. Melanin is a negatively charged, hydrophobic pigment of high molecular weight. Microorganisms like bacteria, *actinomycetes*, and fungi produce biological macromolecules and pigments known as melanin. Eumelanin and pheomelanin are present in the skin of humans and lower organism as well. The word "melanin" was firstly discussed by C. P. Robin in 1873 and later-on the following terminology such as, cells responsible for melanin synthesis in the skin, "the melanocytes", were described. Melanin can be successfully extracted from various plant tissues and animal tissues as well or even chemical synthesis can generate this (C. G. Kumar, Mongolla, Pombala, Kamle, & Joseph, 2011).

1.2 Source and structure

In all living organisms particularly in mammals' skin and hair, a biopolymer which is a naturally occurring substance is presently known as melanin. Its molecular formula is $C_{18}H_{10}N_2O_4$ and has and molecular mass of 318.288 g/mol. It is low soluble in water consisting of the diversified molecule which exists as sulfur-containing yellow pheomelanin and without sulfur dark eumelanin. Eumelanin is of great advantage when compared to pheomelanin because of its more stable nature and is useful in biomedical applications

The molecules under goes biosynthesis process to give rise to different macromolecules with the help of in vivo biomolecules precursors which are formed from catechol molecules. These precursors are formed when l-tyrosine turns into an

intermediate, l-3,4-dihydroxyphenylalanine (L-DOPA), with the help of tyrosinase enzyme and a lso several reactions which form oligomer of 5,6-dihydroxyindole (DHI) and its 5,6-dihydroxyindole 2-carboxylic acid (DHICA).

The physicochemical properties of melanin depend on the ratio of these two molecules (DHI and DHICA) which will also affect the morphology and chelating properties of the metal and thus leading to the final oxidation states. The biochemical supply chain of melanin is mixed. Melanin can be acquired from a wide variety of organic and inorganic species. Most of them are available on a sales form for purchase. Mainly in biomedical field Synthetic melanin are in a great demand, these polymers are chemically synthesized by the mechanism of auto-oxidation and polymerization of phenolic (Tyrosine) or indolic compounds (catechols). Melanin has been reported for highly UV- absorbent potential therefore, the absorption spectra ranges monotonically from 700 to 250 nm. One route is through the catalytic action of tyrosinase on tyrosine or 3,4-dihydroxyphenylalanine (Chadwick et al., 1995).

The largest producers of marine cephalopods, however, Melanin have been well-studied so far, operated by cuttlefish ink known as Sepia as their secondary mode of defence against predators. Plants are yet another potential source, where vegetable melanin can be extracted by a chemical procedure. Various experimental protocols have revealed, raw vegetables containing polymers or monomeric units of flavonoid can be processed to extract melanin from plant cells. Further major source of melanin is microorganisms (bacteria and fungi). The potency of melanin-producing microbes is vigorously determined by their ability to hold bodies together and their affinity to protect themselves from harsh environmental conditions such as high temperature, ultraviolet radiation, and cold burn. Similarly in adverse condition of chemical stress such as reactive oxygen damage., lytic enzymes, heavy metal toxins, and antimicrobials.

1.3 Localization of melanin in cells

Microbial melanin can be located in intracellular and extracellular spaces. Melanized particles isolated from in vitro. Certainly, a phage display antibody to pheomelanin stated that melanin was procured from the septa and extracellular walls of *Alternaria alternata* conidia. In *Colletotrichum lagenarium* and *Verticillium dahliae* melanin is suited in layers and enclosed in the cell wall. In several species like *Sporothrix schenckii and V. dahliae*, melanin are deposited as small granules at the surface of cell wall. In comparison to mammalian melanocytes, where melanin are deposited within specialized vacuoles known as melanosomes. However, In exception microbe such as *Fonsecaea pedrosoi* melanin is accumulated on cytoplasmic bodies comparative to melanosomes and extracellular melanin deposition on cell wall. Fungi may also produce extracellular melanin (Cutler & Swatek, 1969).

1.4 Natural versus synthetic melanin

The melanin which can be naturally obtained from natural sources like plants, animals or microbes etc, is considered to be natural melanin and the melanin which are synthesized in order to produce melanin-like (ML) polymers or nanoparticles using different approaches, is known as synthetic melanin.

One of the approaches is to use an indolic compound catechol having an identical molecular structure to melanin for example dopamine results into Da-quinone and DHI units when oxidized. Polymerization of dopamine results in polydopamine under tris aminomethane base or sodium hydroxide solutions which is the same as naturally occurring melanin granules. This Procedure is similar to the *in-vivo* melanin synthesis approach by utilizing tyrosinase enzyme where the structure of the product obtained is similar to natural melanin. Depending on the water content, melanin has the ability to store electrical charges and polarization so it has good optoelectrical properties. According to this nature natural melanin has a superior water content to that synthetic variant of melanin (Ellis & Griffiths, 1974).

1.5 Melanin production by chemical synthesis

In chemical synthesis, oxidative polymerization of dopamine results in the formation of polydopamine, which has similar properties to natural melanin.

The common utilized methods for synthesizing polydopamine are:

(1) Solution oxidation

(2) Enzymatic oxidation

(3) Electro-polymerization

Solution oxidation involves oxidation with oxygen and self-polymerization of the dopamine monomers under alkaline conditions. The enzymatic oxidation involves the enzymatic oxidation of L-tyrosine with the help of the enzyme tyrosinase. Another method utilizes oxidation of tyrosine, a di-phenolic compound of dopamine, further followed by its polymerization into polydopamine via the action of enzyme called laccase. Electro-polymerization is the method which involves the formation of polydopamine on an electrode. The hydroxylation of L-tyrosine to L-DOPA is catalyzed by the enzyme tyrosinase using molecular oxygen which is then oxidized to dopachrome. Further dopachrome polymerizes to yield melanin nonenzymatically (Ellis & Griffiths, 1975).

1.6 Biosynthesis of Melanin

As per the variety of the molecule in reference to the morphological characteristics, its biogenesis is diverse. The universal pathway applied for the study of melanin synthesis is known as Raper- Mason pathway. This pathway has established the route for eumelanogenesis and later on this pathway has been modified with L-Dopaquinone for the synthesis of pheomelanin. Tyrosinase is an enzyme which catalyses the process of hydroxylation of L-tyrosine which results into L-DOPA via utilizing the molecular oxygen and produces the oxidized compound known as dopachrome. This nonenzymatic polymerization procedure occurs to yield melanin. Based on the sequence of amino acid and their functional features, microbial tyrosinases are categorized into five main groups. The tyrosinase enzyme from *Streptomyces sp*. is indulged in one of these categories. They have common requirement of a molecular chaperone protein which inserts copper atoms into their active pocket of tyrosinase. Melanin is not an essential biomolecule for the particular organisms in response to growth and development, but they play a major output in improvement of their survival rate and competition. Melanin is a phenolic biopolymer with diversified molecular structures carrying negatively charge, hydrophobic; insoluble in aqueous state or any organic solvents, shows resistivity against concentrated acid, and susceptible while bleaching by oxidizing agents. Pigment appears to be black to brownish which is formed by oxidative polymerization of phenolic or indolic compounds. This particular pathway is referred for both eumelanin and pheomelanin synthesis (Fisher & Kripke, 1977).

The quinone is a crucial compound in the animal melanogenesis. By using thiol containing compound such as L-cysteine with L- dopaquinone is way faster then the intramolecular cyclization, which is said to be the pathway for pheomelanin synthesis. The substitution of L-cysteine can take place in any different position to obtain a mixture compound of cys-dopa derivative. This mixture of compounds goes through a chain of reaction to benzothiazines and lead to benzothiazones and finally leads to the formation of pheomelanin polymer. In conclusion, for pheomelanin synthesis availability of free thiol group such as L-cysteine or glutathione is necessary. When there is an absence of the free thiol group, the L-dopaquinone goes to a series of spontaneous intramolecular cyclization reaction which changes into L-leukodopachrome (cyclodopa), for the formation of eumelanin. The main function of the L-leukodopachrome is to enhance the redox reaction with L-dopaquinone (uncyclized form) as it has the strong reducing affinity to regenerate the other half part of L-dopamine and to provide L- dopachrome which is said to be the second crucial step of eumelanogenesis. L-dopachrome is a stable semiquinone which shows absorbance at 305 and 475nm and orange in colour. To study the tyrosinase activity, formation of the L-dopachrome is being determined by the colorimetric assay (Gan, Haberman, & Menon, 1976).

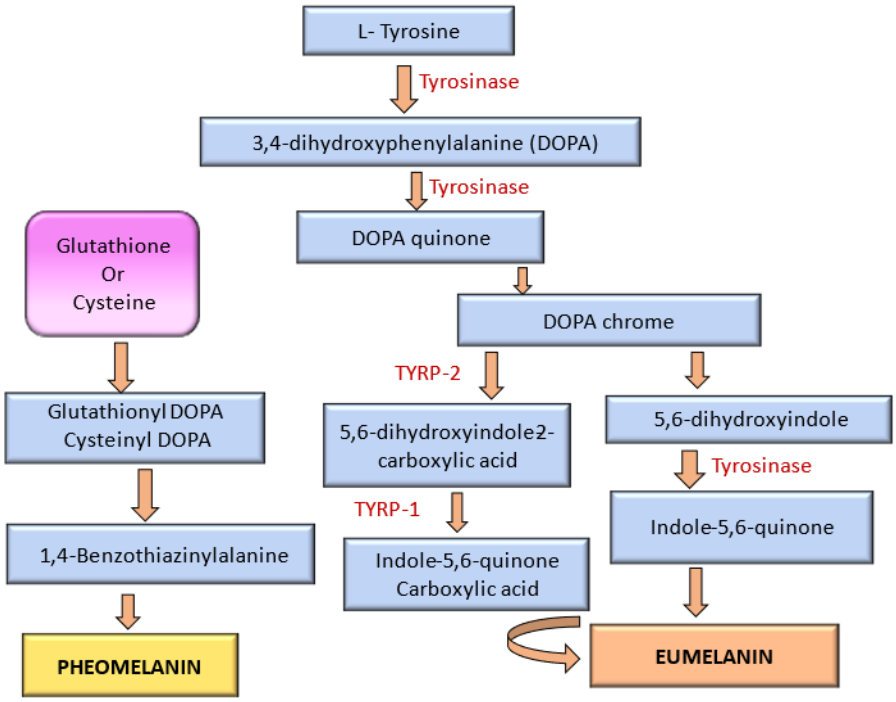

Figure 2 showing biosynthesis of Melanin production by using Tyrosine as a precursor molecule

2. Natural Production of melanin and its application

2.1 Melanin Production in Animals

Melanin is the key pigment of animal body responsible for various pigmentation varying in imperceptible degree creating melanin diversity. Tyrosine is involved in major biological pathways such as catecholamine synthesis and melanogenesis so plays a key role in mammalian life. L-tyrosine is hydroxylated to form L-3,4-dihydroxyphenylalanine (L-DOPA) with the help of tyrosine hydroxylase further L-DOPA decarboxylated to form neurotransmitters such as dopamine, noradrenaline, and adrenaline. L-DOPA and dopamine undergo oxidative polymerization by a series of enzymatic and non-enzymatic reactions to form dark polymer pigments named melanin.

In mammals, there are mainly two types of melanin pigmentation is present, Eumelanin which can be procured via serial oxidation of L-DOPA and dopamine, leads to formation of dihydroxy indole carboxylic acid (DHICA) and 5, 6-dihydroxyindole (DHI) as a copolymer. Generally eumelanin are abundant in mammals specially with the dark coloured pigment containing area like moles, nevi, lengtios and macules. Pheomelanin, in which before its oxidative polymerization, L-DOPA undergoes cysteinylation by conjugation with glutathione or cysteine. The biochemical structure of pheomelanin is still undefined, they are generally present in freckles and nipples. Neuromelanin are also produced from oxidative polymerization in the brain, a mixture of pheomelanin and eumelanin as they are originated from 5-S-cysteinyldopamine and dopamine. Neuromelanin are considered to be the remanent waste of catecholamine metabolism. The data confirmed main function of neuromelanin as a dopaminergic neuron responsible for massive degradation of Parkinson's disease. Melanin is produced by melanocytes mainly found in the epidermal basal layer which is responsible for hair and skin pigmentation. Pigmentation is the main function of melanin in which it protects tissues against mutagenic light. Melanin are also present as a pigment in iris of the eye, similarly in stria vascularis region of the inner ear, and many visceral organs of the body. Recently it has been observed that pheomelanin is almost seen in higher vertebrated including birds, reptiles and mammals. Rest all the lower organisms are found to be rich in eumelanin (Gilchrest, Eller, Geller, & Yaar, 1999).

2.2 Melanin Production in Plants

Working metabolism of plant is depend upon the limiting element on the earth's surface i.e, Nitrogen. Generally, animals, microbes, and fungi uses l-tyrosine as their precursor element to reach the end product, but plants are having different scenario. Melanin synthesis in plants is associated with the enzymatic browning reactions that occur in damaged tissues by polyphenol (Catechol) oxidases which belong to a family of Cu-containing oxidoreductases that are able to act on phenols in the presence of oxygen (Nicolas et al., 1994). The polyphenol oxidases combine with vacuoles of phenolic substrates responsible in forming high reactive *o*-quinones. The *o*-quinones undergoes nonenzymatic polymerization reaction or may interact with other similar compounds, such as thiol groups, amino acids, peptides, and forms-coloured products. *o*-quinones interact with water to form triphenols or can be reduced to the original phenols. Melanin pigments accumulate extracellularly in the form of a pheomelanin layer to exclude plastid-located polyphenol oxidases participation in melanin formation and include phenol-oxidizing enzymes with extracellular localization for melanin synthesis (Wang et al., 2015). Melanin accumulation in chloroplast-derived melanoplasts identified in black grains of barley (Shoeva et al., 2020), As intracellular melanin formation occurs within plastids (Shoeva et al., 2020), and the extracellular Phyto melanin layer occurs as a result of the catabolism of hypodermal cells (Pandey and Dhakal, 2001), it seems that melanin synthesis and Phyto melanin layer formation are different cellular processes that should be distinguished. The overall concept of plant's melanin formation is deficient of nitrogen and is known as allomelanin. As studies have shown in plants tyrosine acts as a poor precursor. The pigment produced in plants is always dark brown to black and their structure is mainly depended on the unit going to be oxidized. In several years, studies in plant melanin have been poorly conducted therefore any defined mechanism has not been reported yet (Nosanchuk & Casadevall, 2003).

2.3 Melanin production by microorganism

The biological roles of melanin in bacteria and fungi have been reported in the literature (Nosanchuk and Casadevall 2003; Plonka and Grabacka 2006; Eisenman and Casadevall 2012; Solano 2014; Cordero and Casadevall 2017). The valuable source of natural melanin is from microbial melanin. The transformation of either tyrosine or malonyl-coenzyme A by different enzymes results in the formation of microbial melanin. This occurs with the help of two pathways Dopa pathway and DHN pathway. The DOPA pathway shows similarity to mammalian melanin synthesis

In L-Dopa biosynthesis pathway, Tyrosine which is a precursor molecule of melanin is changes into L-Dopa and further into dopa-quinone with the help of tyrosinase and laccase enzyme. This dopaquinones will oxidize and auto-polymerized to form melanin due to its highly active nature. This Dopa pathway mediated-melanin synthesis is referred as DOPA-melanin or eumelanin. In the DHN pathway, malonyl-coenzyme A which is the corresponding precursor, is synthesized endogenously. The sequential decarboxylative condensation of five molecules of malonyl-coenzyme A catalysed by polyketide synthases forms 1,3,6,8-tetrahydroxynaphthalene (THN). THN which further goes into a series of reaction called reduction and dehydration to convert into 1,8-dihydroxynaphthalene (DHN). DHN polymerizes to form DHN-melanin. These both DHN and DOPA pathway are found in fungi and bacteria. The synthesis of melanin in most of the bacteria and basidiomycetous fungi occurs via DOPA-pathway whereas in ascomycetous and non-microscopic fungi via DHN-pathway (Ye et al., 2014).

Most of the microbes depend on Tyrosine or its derivatives to form melanin, so melanin act as a main substrate. Apart from tyrosine catecholamines such as dopamine and norepinephrine are also used as substrates. The melanin formed from different substrates will have different structures due to different enzymes and catabolic process involved. The melanin formation highly depends on enzymes regulation in melanin synthesis which is driven by physicochemical conditions. Copper ions play an important role in formation of melanin because they work as cofactor for laccases and tyrosinases enzyme. By the addition of iron and nickel, tyrosinase activity will likely to increase and so melanin production showed by Wang et al. (2019). So apart from copper, few more elements also help in melanin production.

For growing melanogenic microorganisms, there is no such universal culture media or cultivation condition because of diverse factors that affect melanin biosynthesis such as high temperature, nutrient-poor growth media, hyperosmotic pressure etc. So, these factors promote melanin synthesis. Melanin production can be improved by genetic engineering techniques (Brenner & Hearing, 2008).

2.3.1 Melanin synthesis in fungi

Most fungi often produce melanin and the melanin production may protect the living organisms from diverse environmental stress. For example, melanin accounts for 30% of the dry weight of *Agaricus bisporus.* The major part of the human pathogenic fungus are generally environmental organisms which includes many melanotic fungus which plays a vital role in clinical application. Human pathogenic fungus that forms melanin are as follows: *Cryptococcus neoformans, Sporothrix schenckii, Paracoccidioides brasiliensis* and *Scedosporium prolificans* whereas Candida spp. do not melanize.

The fungus is said to contain all the melanin species found. Melanin to mold can be considered as a secondary metabolite product and its appearance can be natural. Although this black pigment may have indirect affect in growth rate and development of the fungus, it can perform a wide range of biological functions such as increased risk for many fungal diseases that affect humans and plants. In molten mold the pigment is mainly appears like a granular form or embedded in thin fibrils on the outer layer or can be intracellularly embedded within cell wall, or binded with chitin cell wall, or extracted without cells. Most fungi produce melanin. e.g., *Aspergillus niger*, *Aspergillus nidulans*, *Alternaria alternata*, *Cladosporium carionii, Fonsecaea compacta, Exophiala jeanselmei, Hendersonula toruloidii,* and *Phaeoannellomyces wernickii* (Caldas et al., 2020).

2.3.2 Melanin synthesis in bacteria

Although tyrosinases occur more frequently than laccases in the bacterial kingdom. Diverse species of *Streptomyces* produce melanin and the production of melanin is described for *Streptomyces* antibiotics and *Streptomyces glaucescens*. Formation of melanin act as a secondary defence response to harsh environmental conditions. Pheomelanin, the brownish to pinkish pigment mainly produced by *Pseudomonas aeruginosa*. Melanogenic *P. aeruginosa* shows major lecithinase enzymatic activity as well as neuraminidase activity which nearly affects their rate of pathogenicity. In *Stenotrophomonas maltophilia*, melanin is synthesized by tyrosinase. *Vibrio cholerae* strain can also form pheomelanin. Melanin formation in *V. cholerae* can be triggered in response to stress, mainly hyperosmotic shock and elevation in temperatures. Melanin can serve as electron acceptors as it is a humic compound (Gessler, Egorova, & Belozerskaya, 2014).

2.3.3 Melanin production in Helminths

Fasciola gigantica has a tyrosinase type of enzyme that exists in both membrane bound and soluble forms and can oxidize mono- and diphenol compounds (Nellaiappan et al., 1989). However, in several varieties of helminths melanin play a crucial role in pathogenesis as showing polyphenol acitivity as a vital factor in formation of egg shell. In *Schistosoma mansoni*, phenoloxidase is associated with cross-linking of precursor proteins in eggshell synthesis (Johnson et al., 1987; Wells and Cordingley, 1991; Eshete and LoVerde, 1993; RibeiroPaes and Rodrigues, 1995). Similarly, *Isoparorchis hypselobagri* has a polyphenol oxidase activity associated with egg-shell formation (Srivastava and Gupta, 1978). In Trichuris suis, phenoloxidase acitivity is located on proximal eggs where it is believed to function in egg shell hardening (Fetterer and Hill, 1993; 1994). For *Trichuris muris* inhibition of phenol oxidase activity by disulfiram results in malformed eggs that are unable to produce murine infection (S. Kumar, Seetharaman, Periasamy, & Raaman, 2008).

3. Melanin isolation from conventional natural sources

The main source of melanin extraction is sepia ink or dark hair/feathers. As most melanin are formed inside melanosomes and they are tightly bound to proteins or minerals so the melanin production and extraction from these sources is one of the great challenges. Therefore, the melanin isolation involves harsh chemical treatments in order to remove the entire protein and such cellular components (Peng, Zhipeng, Fang, & Yiwen, 2020).

These chemical treatments involve strong hydrolysis via boiling mineral acids and bases. The further step is followed by successive washing by using organic solvents such as chloroform, acetone or absolute ethanol to remove the organic contaminants then it suffers chemical alterations.

These involves the use of isolation methodologies such as mechanical separation by ultracentrifugation; proteolytic digestion with the help of enzymes to remove the leftover protein matrix; or a combination of both the technique

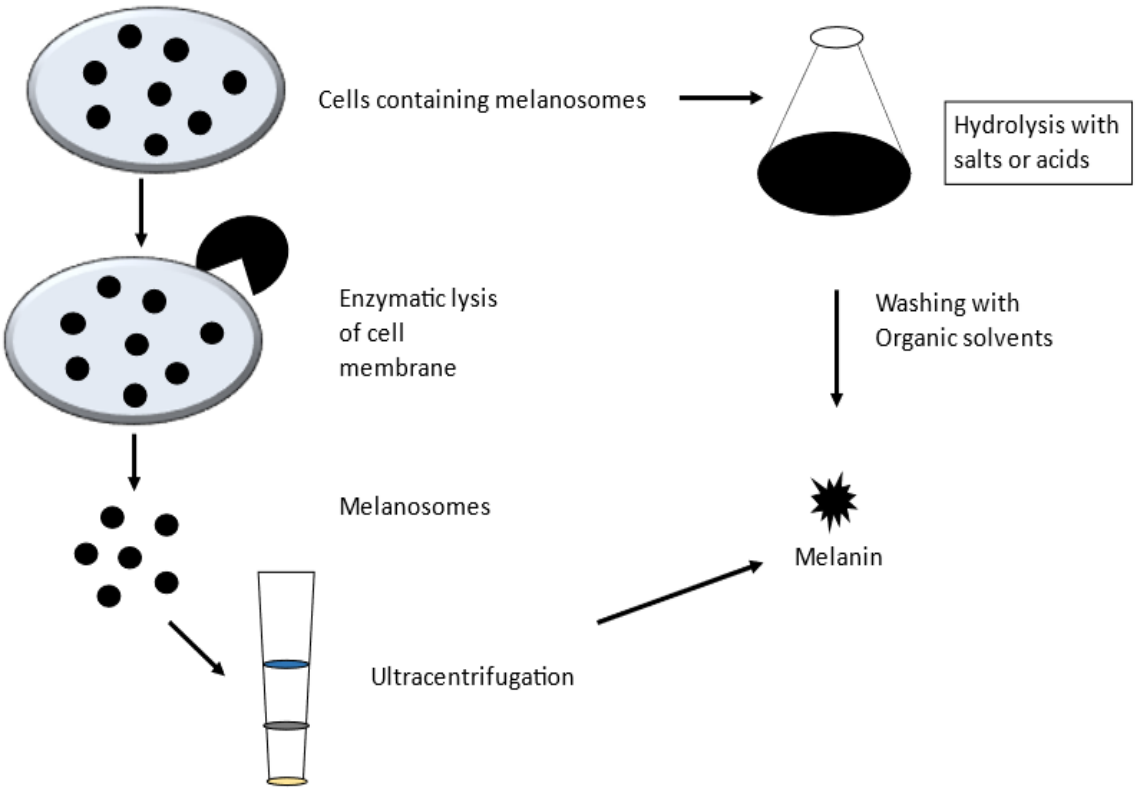

Figure 3 Showing the extraction procedure of Melanin pigment from cell

4. Applications of microbial melanin

One of the major roles of melanin is the virulence of pathogenic organisms in fungi and bacteria.

In fields of green technology, materials science, cosmetics, environmental remediation and biomedicine, melanin pigments can be turned into valuable materials according to the new advancement and technologies. Melanin is a strong antioxidant so it helps in blocking UV light so act as a natural "sunscreen" which absorbs UV–visible light spectrum's broadband. The important advantage of melanin are its biodegradability, bioavailability and biocompatibility so it has a promising role in biomedical applications. It also shows hydration dependent semiconductor-like behaviour. It also helps in the synthesis of silver nanostructures. These silver nanostructures show anti-microbial activity against food pathogens so it is useful in food industries as well as health industries. Melanin are metal chelators so helpful in environmental applications. According to cosmetic industries melanin shows its applications in use for sunscreen and hair dyeing.

Melanin compounds have extensive antimicrobial activity, antitumor, antivenin activity, anti-virus, hepatoprotective activity. It helps in protecting living organisms from extreme high or low temperature and many carcinogenic radiations such as ultraviolet rays. It can also be utilized in sunscreen lotions and ointments because of their absorbing nature and scavenging properties. It helps in cosmetic property in minimizing premature ageing by protecting the cells from high energy visible light.

Streptomyces strains use tyrosinases for the synthesis of melanin pigments. Melanin and its biochemical derivatives can be further explored as a strong therapeutic agents against retinitis pigmentosa, various neurodegenerative diseases like Alzheimer's, dementia and schizophrenia. The melanin pigment is an eco- friendly dye for dyeing and printing of wool fabrics which serves as a replacement of synthetic dyes (Guindre-Parker & Love, 2014).

Majority of achivements listed by melanin nanoparticles (MNPs) in biomedical field briefing the antioxidative property and photoprotective effect.

Applications	Sources	Production Methodology	Size of particle	Output
Antioxidant analysis and photoprotective activity.	Dopamine Hydrochloride Sepia officinal's ink. Synthetic	Oxidation Washing and purification Sol-gel enzymatic polymerization oxidation	<100 50-300 <80 <70-80	• Hela cells with good biocompatibility and free radical scavenging activity. • Primary human keratinocytes internalisation. • Reduced amount of reactive oxygen species and reactive nitrogen species. • Avoidance of typical tendencies of aggregation and uncontrolled particles growth efficient crytoprotective effect protection of bone marrow from ionising radiation. • Radioprotection is effective against radiation.
Drug delivery	Lactosylated Dopamine Dopamine hydrochloride	Oxidative polymerization Oxidative and self-polymerization. Washing and purification.	<150 <100 100-250	• Absorbance of light is strong near-infrared. • Loading capacity of drug is 66-73 wt%. • Targeting effect is active (tunable) • Chemo-photothermal activity shoes synergistic effect. • Drug's Loading capability is distinct and protects from unresponsive leakage in physiological environment. • Retaining capacity of drugs. • Good stability was released by multi-responsive controlled drug. • Human body's widely biodistribution. • Near-infrared absorption is strong and efficiency of

				photothermal conversion is high. • Antibiotics nano delivery system. • Possibility of loading functionalized nanoparticles onto various bioactive molecules.
Imaging	Synthetic Dopamine hydrochloride	Stirring Oxidative and self polymerization.	<10 <50	• In-vivo tumour imaging property is good. • Magnetic Resonance Imaging Agents for Tumour Target Specificity. • optoacoustic tomography contrast agent • Good dispersion stability • of high concentrations of melanin nanoparticles in biological media • Lower cell toxicity than that of gold nanorods.
Theranostics	Black sesame seeds Dopamine hydrochloride Sepia officinal's ink Synthetic	Washing and purification Washing and purification Oxidative and self polymerization Vigorous stirring co-precipitation.	20-200 <200 <200 <200 <10 <150	• Lack of long-term toxicity in mice • Photothermal effect significa ntly suppresses tumour growth. • Strong near-infrared absorption and high photothermal conversion efficiency. • Blood pooling and effective accumulation at the tumour site. • Near-infrared light absorption that greatly improves photothermal therapy treatment. • treatment efficiency. • Photoacoustic agent of imaging. • Contrast MRI improvement. • Promising multimodality-

				imaging guide therapy. • Good photoacoustic intensity of signals. • Absorbance of near infrared region is effective. • Effective treatment of photo-thermolysis.
Bioelectronics and bioengineering.	Sepia officinal's ink Dopamine hydrochloride Synthetic	Washing and purification Layer by layer Oxidative and self-polymerization Self stirring	<200 <200	• Electro-chemical's conductivity is durable. • Decreased secretion of pro-inflammatory cytokines • Antibacterial properties. • Development of MNP-based adhesive interfaces for bio-adhesive surgical membranes. • Efficient conversion of protons to electron currents. • Operation by so-called volume gating. Premises for further miniaturization

4.1 Cosmetic applications

4.1.1 Sun screening and radioprotective effects of melanin

Melanin from natural sources possess various biological activities, which include UV radiation protection, enzymatic lysis, oxidants damage, drug resistance pathogens, protection of insects against bacteria and antiviral protection. The use of melanin in cosmetics and sunscreens has been reported by many manufacturers. Sun Protection Factor (SPF) scale indicates the level of protective effect of sunscreen so higher SPF value shows better protective capacity. The SPF of 2 represents the duplex of defence power of particular skin against cold-burn or sunburn. Melanin-containing mushrooms can provide significant protection against radiation so developed as radioprotectants reported by Revskaya et al. Valavanidis et al. used free radicals' mechanisms in UV-induced skin photocarcinogenesis by electron spin resonance (ESR).

Determination of Skin colour mainly depends on the amount of oxy-deoxy-haemoglobin, presence of carotenoids and types of melanin and also arrangement of melanin in melanosomes. The melanin formation usually occurs in melanosomes that are formed in dendritic melanocytic body. Melanin is composed of complex soluble red / yellow, alkali sulphur-containing pheomelanin and brown / black insoluble eumelanin. Skin pigmentation Differences results from variation in their melanogenic activity and the particular melanin formed in melanosomes but not differ in their number of melanocytes. Eumelanin formation takes place in elliptical melanosomes, whereas formation of pheomelanin occurs in smaller round melanosomes. Other important properties of eumelanin are its functions as a free radical scavenger and superoxide dismutase that reduce ROS (Sun et al., 2016).

4.1.2 UV and pigmentation

An increase in melanogenesis is induced by UV-induced DNA damage and its repair which is produced produce signals after UV irradiation. Melanin is a strong antioxidant so it helps in blocking UV light so act as a natural "sunscreen" which absorbs UV–visible light spectrum's broadband. UV-exposed melanocytes are treated by the enzyme T4 endonuclease V which is responsible for increased DNA repair as well as melanin content. Thymidine dinucleotide (small DNA fragments) effect serve as a model for thymidine dimers. In vitro, increase in melanin content and increase in tyrosinase mRNA was observed after treatment with Thymidine dinucleotide which is responsible for melanin synthesis (Bharathi, R, & Jayalakshmi, 2011).

4.1.3 Photoprotective role of melanin:

Role of melanin as a photoprotective agent shows inverse correlation in comparison to pigmentation of skin and the chances of radiation-induced skin cancers. Melanin mostly eumelanin shows shielding effect by its strong affinity to play a role of physical barrier which emits UV radiation, act as strong absorbent which helps in reducing UV penetration through the epidermis. So dark skin is better protected against UV-induced damage than fair skin which has less eumelanin. Melanin absorbs 50–75% of UV radiation so its sun protective factor is about 1.5 to 2.0. So, eumelanin is superior in its photoprotective properties than pheomelanin. Other fundamental properties of eumelanin are their key role in free radical scavenging and superoxide dismutase which decreases ROS. Melanosomes in dark skin remain intact and are not degraded throughout the epidermal layers which contribute to photoprotection against UV-induced damage by forming supranuclear caps in keratinocytes and melanocytes while in lightly pigmented skin melanosomes are degraded (Zou, Hu, Ma, & Tian, 2015).

4.2 Biomedical applications

4.2.1 Antioxidant effects of melanin

Melanin shows its antioxidant properties because of its affinity to diminish excited state molecules like pigments, free radicals scavenging and bind strongly to metal ions to produce melanin-like nanoparticles, by hydrochloride oxidation. These melanin-like nanoparticles have constant electron spin resonance (ESR), which shows the stability of free radical canters. In performing radical scavenging property of the melanin-like nanoparticles were studied by an 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Due to the importance of melanin in wound healing and skin repair, they extracted melanin nanoparticles from the cuttlefish ink known as *Sepia officinalis*, and incorporated in a compound like gellan gum to achieve spong-like hydrogels to control sustained release of melanin conjugated nanoparticles. This conjugation would play an important role in various skin disease. To evaluate cytotoxicity and antioxidant properties, melanin nanoparticles were released into human primary keratinocytes cultures, at different amounts. Through the reduced amount of ROS released by UVA/UVB irradiated human primary keratinocytes, the antioxidant properties were observed and through the internalization of eumelanin nanoparticles by primary human keratinocytes, photo protective behaviour was observed. Extracts obtained from berry of *Cinnamomum burmannii* and *Osmanthus fragrans* includes antioxidant melanin that also carry metal-chelating activities. Melanin has potential applications as a natural antioxidant in the cosmetic and pharmaceutical industries and also have free radical scavenging activity, which is showed by Kumar et al. by the antioxidant activity and physicochemical characterization of melanin from a strain of *Aspergillus bridgeri* studies (Cragg, Chadwick, Potten, Sheehan, & Young, 2002).

4.2.2 Anticarcinogenic effects of melanin.

In 1997, Kamei et al. showed that the growth of HCT-15 cells and Meth/A cells has been suppressed by the peptide-free allomelanin obtained from soya beans and sesame seeds which are showing black pigment. The addition in high concentration of protein-free allomelanin to HCT-15 culture medium blocked the S phase of the cell cycle using flow cytometry. Offen et al. showed experimentally, that synthetic DOPA-melanin is responsible in causing 50% apoptosis in the PC-12 cell line. Based on this study the role of melanin in inducing apoptosis in PC12 cells has been suggested. Blinova et al. studied the effect of 19 melanin preparations isolated from black yeast fungi on keratinocyte and fibroblast proliferation (Young, Potten, & Sheehan, 2002).

4.2.3 Anti-inflammatory effects of melanin

Administration of melanin which is extracted from *Ommastrephes bartrami* LESUEL on carrageenan-induced rat oedema by intravenous or intraperitoneal injection, suppressed the induced acute and subacute inflammation has been reported by Mimura et al. Avramidis et al. (1998) studied the effect of grape melanin on carrageenan-induced oedema interferes with the prostaglandin, leukotriene that mediate inflammation, thus showing a remarkable inhibitory effect.

The role of melanin pigmentation in gingival inflammation has been studied by Nilima et al. in pigmented and non-pigmented groups. The pigmented group showed lower markers of inflammation Compared with the non-pigmented group, like gingival index and bleeding index, which shows the higher activity of melanin as protective agent against gingival inflammation. The anti-inflammatory effect of *Nigella sativa* L melanin has been studied by El-Obeid et al. (2016) against formalin-induced rat-paw oedema. The application of melanin shows strong anti-inflammatory action (Tewari, Lahmann, Sarkany, Bergemann, & Young, 2002).

4.3 Bioengineering and bioelectronics applications

Melanin is used as a nanocarrier for bioengineering applications due to its internal conductivity, act as metal chelating agent and having biocompatible properties. Melanin nanoparticles shows conjugation with poly (vinyl alcohol) to produce a nanocomposite film. In bioengineering, biomaterial can be used into electroceuticals and implantable devices with pronounced improved features. With respect to approach of wound healing, the majority materials used are foams, gauzes and hydrogels that are applied to prevent antimicrobial infections. Melanin nanoparticles biocompatibility were assessed by enzymatic activity of lactate dehydrogenase (LDH) assay, using dermal fibroblasts as precursor, which exhibit antibacterial effects against gram negative and positive bacteria. Concerning bioelectronics, the major challenge is to connect ionic signals with conventional electronics, which allows the creation of new medical therapies. Sheliakina et al. demonstrated a transducing interface based on conductivity of melanin and a p-type organic electrochemical transistor. Melanin was synthesized and used for film casting (Paproski et al., 2015).

4.4 Nanomedicine applications

Melanin-like Nanoparticles are superior in the field of nanomedicine compared to many inorganic nanomaterials due to their biocompatibility and photothermal properties. Melanin like Nanoparticles, Outstanding photothermal conversion ability make attractive platform for the development of biomedical applications. Other important applications of melanin-like Nanoparticles are in biomedicine field including diseases treatment and biological imaging platform The inherent photoacoustic imaging ability of melanin-like NPs has great applications in disease diagnosis and efficacy evaluation. As melanin-like NPs has strong metal chelation ability, it can be explored for detection and imaging technologies similar to magnetic resonance imaging (MRI) and positron emission tomography (PET) to reflect function of tumour cells accurately and metabolic information. Melanin-like Nanoparticles can be used for multi-modal imaging in some studies in order to obtain complementary diagnostic information (Caldas et al., 2020).

4.5 Applications of Melanin in Medicine

One of the important advantages of melanin is that it is present within our human body in melanocytes as nanosized particles. Melanin nanoparticles is a safe biopolymer for biomedical field applications due to their biocompatibility nature. Melanin pigments can be used for the clinical development of drug delivery systems, imaging, or theragnostic as it has unique physicochemical properties. Melanin has the ability to bind molecules and absorb light so can be used as a drug carrier. Drug-loaded melanin-like nanoparticles can efficiently target cell organelles and mitochondria. Melanin is a photosensitizer, because of its ability to produce heat when they are exposed to light energy. Sensitized heating of melanin within the tissues can be explored in photothermal therapy which is a great approach to kill specific cells without affecting healthy surrounding tissue. Wavelengths of infrared region useful for therapeutic applications minimum absorption seen by tissues in this spectrum region enhancing penetration and targeting efficiency. Melanin is a biocompatible and non-toxic agent when compared to other organic agents. Photo-thermal therapy which is melanin based is useful in the field of cancer immunotherapy as it can directly kill tumour cells, hence helps in promoting tumour antigen release and ultimately immune-mediated anti-tumour responses(Peng et al., 2020).

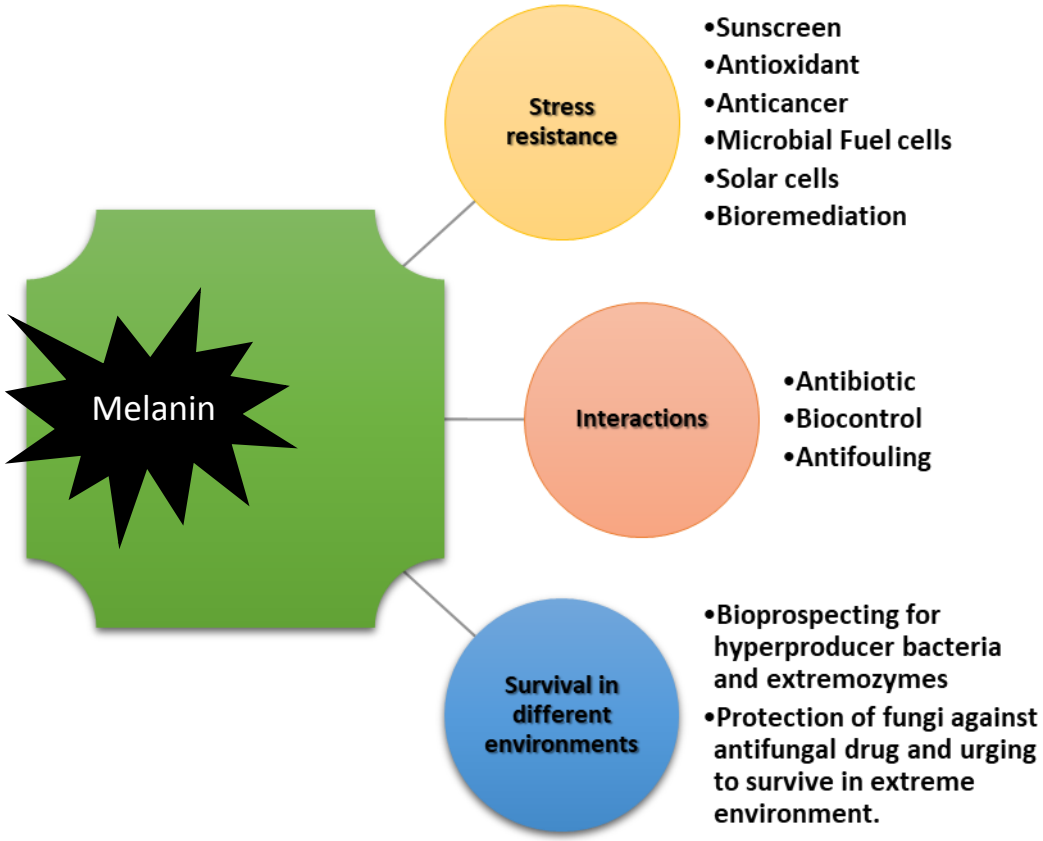

Figure 4 Showing application of melanin in broad spectrum.

5. Deleterious effects of melanin

Melanin can be considered to be a potential molecule against radiations like UV-induced cancerous cells and photodamage. Melanin may also have least toxic effects in response to certain conditions. It reacts with DNA and act as a photosensitizer that produces ROS after UVA radiation. Pheomelanin is prone to photodegradation in contrast to eumelanin. Pheomelanin has damaging effects of ultraviolet radiations because they can generate hydrogen peroxide free radicals and superoxide anions which are responsible for mutations in melanocyte or other associated cells with apoptotic activity. It also helps in increasing the release of histamine levels which contributes to the sun-induced erythema and edema. It also causes cell death as it is a UVA and UVB sensitizer. Thus, it is possible that pheomelanin has a weak carcinogenic effect that can contribute to melanoma formation (Bull, 1970)(Ellis & Griffiths, 1975).

6. Gaps and challenges

Briganti et al., 2003 reported overproduction of melanin occur with chronic sun exposure, melasma, or other hyperpigmentation diseases. Pierluigi et al., 2003 reported, when free radicals are improperly processed during melanin synthesis, hydrogen peroxide (H2O2) is formed, producing hydroxyl radicals and other reactive oxygen species. Seiberg et al., 2000 concluded melanin biosynthesis can be inhibited by avoiding UV exposure, inhibiting melanocyte metabolism and proliferation, inhibiting tyrosinase, or removing melanin by corneal ablation. Kadekaro et al., 2003 demonstrated the use of tyrosinase inhibitors to maintain skin whiteness, increasingly used in cosmetics. Yamakoshi et al., 2003 -A role for oxidative stress in the pathogenesis of skin diseases has been demonstrated. Ma et al., 2001 reported that inhibitors such as ROS scavengers or antioxidants can reduce hyperpigmentation. Traditional herbal medicines for the development of new skin-care cosmetics have been emphasized recently (Kiken and Cohen, 2002). In this study, the anti-tyrosinase effects of 95% ethanolic extracts of several traditional Chinese herbal medicines used in antiquated skin care books were evaluated in

human melanocyte cultures. Their antioxidant capacity and phenolic content were also tested. Siva Deepthi Seelam demonstrated the characterization and photoprotective potential of calcareous Pseudomonas-mediated melanin as a sunscreen against UV-B radiation. Radames J. B. Cordero et al reported microbial melanin for radioprotection and bioremediation. NY Yoon et al examined 7-phloroeckol may prove useful as a new inhibitor of melanogenesis in cosmetic applications. Yu Zou et al showed the physicochemical Properties and Antioxidant Activities of Melanin and Fruiting Body Fractions of Auricularia Auricula. P. Selvakumar et al did research on the isolation and characterization of melanin pigment from Pleurotus cystidiosus (telomorph of Antromycopsis macrocarpa). In 2011, Huang et al. demonstrated Addition of melanin extracts from Cinnamomum burmannii and Osmanthus fragrans increased the SPF value of gel formulations. Revskaya et al. (2012) investigated the possibility of creating a radioprotectant based on melanin using edible black mushrooms. Kunwar A et al. (2012) studied the putative mechanism of the radioprotective effect of extracellular melanin (isolated from the fungus Gliocephalotrichum) in mice subjected to 7 Gy total body irradiation. Lei et al. (2008) conducted an interesting study on the use of a melanin–iron complex to induce remission of iron-deficiency anemia. Seniuk et al. (2011) [16] reported various favorable effects of melanin complexes on pure cultures of Helicobacter pylori, Candida albicans, Herpes vulgaris I, and HIV-1, both in in vitro and in vivo animal models. Davide Liberti studied Melanin-related phenolic polymers with strong photoprotective and antioxidant activity for dermocosmetic applications. Gloster and Neal showed that melanin is twice as effective at blocking UVB radiation transmission in dark skin compared to fair skin (70). The role of melanin as a scavenging or quenching molecule for superoxide anions and singlet oxygen species was discussed by Tada M et al, (2010). Hoogduijn et al. (2003) [20] observed that melanin protects melanocytes and keratinocytes from DNA damage caused by hydrogen peroxide, indicating that the pigment has an important antioxidant role in the skin. The physicochemical characterization and antioxidant activity of melanin from a strain of Aspergillus bridgeri have been studied by Kumar et al. (2011), who demonstrated that melanin exhibits significant free radical scavenging activity. This research suggests that melanin may have potential uses as a natural antioxidant in the cosmetics and pharmaceutical industries.

7. Remarks and Future perspectives

Next scenarios such as artificial intelligence, recombinant microbes, and green nanomelanin synthesis are other approaches to synthesize melanin. Finally, future prospects need to pay more attention to the above topics for saving melanin production by using agro-industrial by-products as an environmentally friendly alternative. Research should focus more on the semi-industrial production of melanin using simple microbial culture processes, thus avoiding the use of purified tyrosinase, expensive chemical processes, and laborious extraction of polymers from plant and animal tissues.

Future investigations should focus further on melanin pigments and the pharmacological activities of their NPs. This will be of great help in developing new strategies for the treatment of some diseases such as cancer. Melanin is useful in biomedical applications, such as antioxidants, drug extractions, photography, bioelectronics, diagnostics and therapeutics, and cosmetic applications. The results are very promising, leading to a new era of medicine and cosmetics. Melanin is a natural pigment that can be considered an active ingredient in many industrial applications. The future of melanin-based products and technological advances rests on the ability to produce melanin of chemically defined structure in large quantities at low cost. Although there are a variety of active ingredients with photoprotective properties, from antioxidants to plant extracts of DNA repair enzymes, it may be desirable to better understand melanin, its photoprotective properties, and the contribution of melanocytes to cancer.

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