

A Comprehensive Review of Plant Melanin: Synthesis, Phytochemicals, and Herbal Medicine Relevance

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Abstract

This extensive collection of research delves into different aspects of melanin, shedding light on its versatile nature and potential uses. Encompassing a broad range of topics, these studies explore the use of synthetic melanin nanoparticles for tumor treatment, the improvement of melanin extraction from natural sources, and provide evaluations of extraction techniques, microbial melanin, and physicochemical properties from various origins. The research also investigates the potential benefits of using melanin in health food and food additives, as well as its role as a protective agent against sun damage. By highlighting its unique properties, such as its antioxidant effects and neuroprotective qualities, these studies provide a comprehensive understanding of melanin, including its classification, structure, biological activity, and potential applications in different fields. The compilation includes abstracts of studies that focus on the production of plant melanin, with particular attention to genetic processes and the significance of polyphenol oxidases in *Trichomerium bhatii* NFCCI 4305. It also examines the connection between skin pigmentation and health risks, indicating a higher prevalence of migraines in individuals with lower levels of melanin. Additionally, an alternate approach for synthesizing melanin is presented.

Keywords: Melanin, nanoparticles, extraction optimization, antioxidant properties, green synthesis, skin health.

Introduction

The concept of "Melanin" is derived from the Greek word "Melanos," meaning dark or black, referring to the appearance of the pigment. In 1840, Swedish chemist Berzelius first used this term to describe a dark pigment extracted from eye membranes (Borovansky 2011). Melanin has a long history, existing naturally since the beginning of life on Earth. It has been found in well-preserved dinosaur fossils, feathers of ancient birds, prehistoric marine creatures, and even in bacteria and fungi from ancient times. Melanin is a diverse group of natural pigments that vary in origin, color, composition, and functions in different organisms (Juhas et al. 2021).

In humans, melanin is primarily responsible for the coloration of skin, hair, and eyes (Hushcha et al. 2021), with the exception of Neuromelanin, found in the central nervous system and does not affect pigmentation (Zecca et al. 2001, Ishikuro et al. 2021). The Ebers papyrus (1550 BC), an ancient document on Egyptian medicine, mentioned skin diseases related to skin color, possibly referring to vitiligo, a term derived from the Latin word "vitellus," meaning "veal" or pale pink skin.

The earliest written account of skin pigmentation in humans is attributed to the Greek historian Herodotus, who noted the darker skin tones of Persians, Ethiopians, and Indians compared to Greeks, providing an important historical reference for understanding diversity in skin color (Ebbell, 1937). The most common belief in Europe and Arabic civilizations was that dark pigment was derived from the decomposition of haemoglobin. However, the first chemical analyses by Berzelius and others showed significant differences between melanin and haemoglobin, making this relationship unlikely (Borovansky 2011).

Recent research has focused on the properties of melanin extracted from sunflower seed shells, highlighting its potential as a natural colorant, antioxidant, and adsorbent. Physicochemical tests, such as UV-vis spectra and DPPH scavenging activity, have shown its

antioxidant properties. This suggests that melanin from sunflower seed shells could be a promising alternative to synthetic melanin due to its effectiveness in various applications.

For a comprehensive analysis of the specific properties of this natural melanin, the original article titled "Isolation and Biological Activity of Melanin from Sunflower Seed Shell" in *China Biotechnology*, 2022 provides detailed information. In animals and plants, melanin is responsible for the coloration of seeds, berries, flowers, human skin, and hair. It is also used commercially in photoprotective creams and anti-melanoma therapy and has been reported to possess immunopharmacological properties (Montefiori and Zhou 1991). Melanin also has high commercial value in the food industry as a natural additive and colorant. As the demand for natural colorants and additives increases, the use of synthetic colorants is often perceived as undesirable or harmful. Plants are an excellent natural source of pigments, including melanin (Wang et al. 2006).

To fully understand the complexities of melanin, it is essential to explore its concept and structure. Based on their sources, melanin can be classified into five main types: animal melanin, plant melanin, fungal melanin, bacterial melanin, and synthetic melanin. These distinctions arise from significant variations in the phenolic units of the polymer and the characteristics of oxidases necessary for polymer formation (Solano, 2014).

As we examine different sources and extract melanin, specific names are used, such as eumelanin, pheomelanin, neuromelanin, allomelanin, and Pyomelanin. However, establishing a consistent correlation between the source and types of melanin is a challenging task. In terms of plant melanin, there is a noticeable gap in research compared to animals and microorganisms. This is due in part to the complex polymeric nature of the pigment.

Additionally, plant melanin tends to accumulate in seed coats, alongside compounds with similar colors like proanthocyanidins. As a result, the biochemical and molecular genetic aspects of melanin formation in plants have received less attention. Any study in this area must first confirm the melanic nature of the pigment. This review aims to evaluate the current state of research on plant melanin, with a focus on its structure, function, localization, synthesis, and relevance to phytochemicals and herbal medicines. It should be noted that our emphasis will be on studies where the melanic nature of the pigment has been validated through rigorous phytochemical and herbal methods (Solano, 2014).

Melanin, a multifaceted biological pigment, plays a pivotal role in various living organisms, providing photoprotection and enabling survival in harsh conditions. Its extraction, however, poses challenges due to varying procedures across different cells and tissues, with bacteria offering a more straightforward isolation process. The corrosive nature of acids and alkalis in conventional extraction methods raises economic feasibility concerns, urging exploration of alternative techniques like TBAOH extraction (Noble K Kurian, unknown).

The TBAOH extraction method offers a promising alternative to conventional procedures for isolating melanin. By avoiding the use of corrosive acids and alkalis, this technique addresses economic feasibility concerns. Furthermore, it provides a valuable avenue for studying melanin's polymerization mechanisms, physicochemical properties, and potential applications in various fields (Luyao Tian et al., 2022).

The research conducted by Nazish Khan et al. on *Pinus wallichiana*-synthesized silver nanoparticles adds to the growing body of knowledge on nanoparticle synthesis using natural sources. Their study highlights the safety, antipyretic effect, and antibacterial activity of these nanoparticles against *Acinetobacter baumannii*. This research contributes to the understanding of sustainable and eco-friendly methods for nanoparticle production.

Additionally, Ioana-Ecaterina Pralea et al.'s work on melanin analysis provides a comprehensive overview of the diverse properties and functions of melanin. Their research justifies the ongoing interest in studying melanin and contributes to the development of evolving methodologies in this field. By exploring the challenges and advancements in

melanin analysis, this study paves the way for further research and innovation in understanding the potential applications of melanin.

In the realm of plant biology, the presence and functions of melatonin unveil a fascinating narrative. Melatonin in higher plants, explored by Jan Kola and team, not only contributes to delaying flower induction but also serves as an antioxidant, exhibits auxin-like effects, and acts as a signaling molecule in interactions with herbivores and pests. Despite extensive research, there is still a need for further exploration to fully understand the diverse roles and effects of melatonin in plants (Jan Kola et al., 2005).

The intricate relationship between ultra-fine grinding of plant raw materials and melanin extraction kinetics is examined by Igor Lomovskiy and colleagues. The study delves into the effects of mechanical treatment on polyphenol extraction kinetics, employing advanced mathematical models such as the Baker equation and Lonsdale model. Focusing on *Ganoderma applanatum* and buckwheat husk, the study underscores the significance of considering particle shape-factor in models based on Fickian diffusion for adequate extraction kinetics characterization (Igor Lomovskiy et al., 2021).

Unravelling a distinct connection between melanin levels and migraines, Magdalena Kobus and team conducted a population-based case-control study. Their findings reveal that individuals with a low melanin index exhibit a more than threefold increased risk of migraines, emphasizing the association of fair skin, resulting from lightly pigmented skin, with migraine prevalence. This study pioneers the investigation into the relationship between skin pigmentation and migraine risk (Magdalena Kobus et al., unknown).

In the realm of natural melanin extraction, Xin Liu and collaborators isolate and characterize melanin from *Auricularia polytricha*. Their work highlights the melanin's solubility properties in alkaline solutions, low solubility in distilled water and organic solvents, stability to most metal ions, and significant antioxidant activity (Xin Liu et al., 2019). Similarly, Noura El and colleagues delve into the current trends and future approaches related to natural melanin, with a specific focus on microbial sources, contributing to the evolving landscape of melanin research (Noura El et al., 2022).

F. Solano's comprehensive exploration sheds light on melanin as a ubiquitous, resistant, and ancient pigment found across various organisms. The biochemistry of melanin involves enzymatically-controlled phases and uncontrolled polymerization of oxidized intermediates, leading to extensive research in fields like dermatology, biomedicine, cosmetics, microbiology, and fruit technology (F. Solano, 2014).

Exploring the therapeutic potential of bacterial melanin, Tigran Petrosyan and team demonstrate its ability to cross the blood-brain barrier and reach specific regions within the central nervous system. The study suggests saturable transport and potential neuroprotective effects, shedding light on the enzymatic stability of bacterial melanin in blood and brain tissue (Tigran Petrosyan, unknown).

Chaofeng Li et al. investigate the antioxidant potential of melanin from sunflower testae, positioning it as a promising natural antioxidant with potential applications across various industries (Chaofeng Li et al., 2018). Chang-Jun Liu's review provides a holistic perspective on melanin in plants, linking it to enzymatic browning, genetic processes of synthesis, and potential roles in wound response, offering insights for future research (Chang-Jun Liu et al., 2020).

Julio Montes-Avila's work on melanins from *Vitex mollis* and *Randia echinocarpa* introduces potential photoprotective agents with antioxidant properties. Stability under artificial light, bleaching under sunlight, and reactions to various oxidants highlight the dynamic nature of these melanins (Julio Montes-Avila et al., 2018).

The study on melanin from *Trichomerium bhatii* by Malika Suthar et al. underscores its suitability for cosmetic products due to its good antioxidant activity. Falling into the DOPA

category and exhibiting characteristics akin to synthetic DOPA melanin, it presents opportunities in derma-cosmetic industries (Malika Suthar et al., 2023).

Advances in the green synthesis of nanoparticles, as explored by Aman Gour et al., emphasize the significance of utilizing plant metabolites to reduce toxicity, opening avenues for applications across various fields (Aman Gour, 2019). Additionally, Ghada Khouqeer et al. delve into the non-crystalline nature of melanin and its nanocomposite formed by copper doping, showcasing enhanced magnetic properties and potential applications (Ghada Khouqeer et al., 2022).

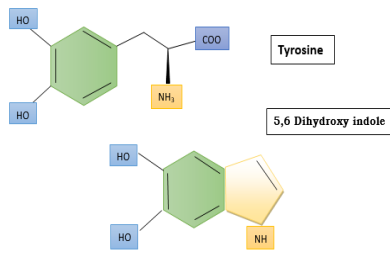
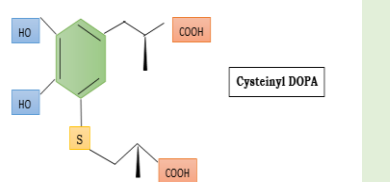
Marino B Arnao and team shed light on the potential of Phyto melatonin as a nutraceutical, presenting diverse protective effects in plants and potential health benefits for humans. Its modulation of circadian rhythms, mood, sleep, and antioxidant properties position Phyto melatonin as a promising avenue for cancer treatment and overall health (Marino B Arnao et al., 2018).

Saranshu Singla et al.'s investigation into melanin extracted from black knots reveals irregular morphology and broadband UV absorption, confirming its identity as allomelanin. The study suggests its potential use for UV protection across various applications (Saranshu Singla, 2021). Muneeba Zubair Alam and colleagues explore melanin in date palm fruits, emphasizing its high levels and potential dietary impact. The study provides evidence of melanin's nature through various analytical methods, contributing to the understanding of melanin in dietary sources (Muneeba Zubair Alam et al., 2022).

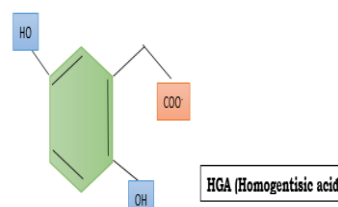
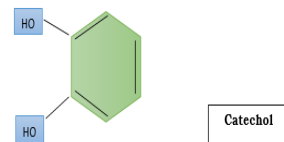
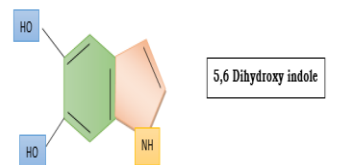
Types of Melanin

According to Solano (2014) and Singh et al. (2021), melanin is a type of amorphous aromatic polymer that is formed from phenolic or indole compounds. Its structure consists of smaller molecules, and the composition and bonding patterns of these molecules greatly impact the type of melanin produced. The prevalent classification of melanin includes eumelanin, Pheomelanin, allomelanin, neuromelanin, and Pyomelanin, as listed in table 1 (d'Ischia et al., 2013).

Table 1. Five major melanin types and their precursors adapted from d'Ischia.

S. No	Pigments	Monomer precursors	Chemical formula	Diagrams
1.	Eumelanin	Tyrosine, 5-6-dihydroxyindoles	Tyrosine, 5-6 dihydroxy indoles	
2.	Pheomelanin	Cysteinyl dopas, Benzothiazines	Cysteinyl DOPA	

3.	Neuromelanin	Dopamine, Catecholamines	5-6-Dihydroxy indole
4.	Allomelanin	1-8-Dihydroxynaphthalene, Phenolic precursors	Catechol
5.	Pyomelanin	Homogentisic acid	HGA (Homogentisic acid)



Eumelanin is responsible for the dark pigmentation of human hair and skin, as well as being produced by certain bacteria and fungi. This pigment has a vital role in protecting the skin from the damaging effects of UV radiation from the sun, preventing DNA damage and skin cancer. It also determines the skin color and provides natural protection against sunburn. In hair, eumelanin is responsible for the wide range of hair colors, depending on the amount and type present in the hair shaft. Furthermore, eumelanin also helps maintain the health of hair by protecting it from UV radiation. The relationship between eumelanin and skin color is primarily determined by the amount, distribution, and type present in the skin. Melanocytes, specialized cells, produce two forms of eumelanin: brown eumelanin and black eumelanin. It is produced from the oxidation of tyrosine and/or phenylalanine into L-3,4-dihydroxyphenylalanine (L-DOPA), which is then transformed into dopachrome and ultimately eumelanin. A study on a marine sponge containing eumelanin suggested it as a potential source for extraction.

Pheomelanin, present in advanced creatures like mammals or birds, is composed of sulfur-containing units, such as benzothiazine and benzothiazole. It is a red, or unheroic, color derived from tyrosine, and is more prevalent in female birds with raspberry-colored feathers. However, too much melanin can be harmful and can lead to skin cancer, as it produces reactive oxygen compounds when exposed to UV radiation. Even after hours of UV exposure, DNA damage can still occur in melanocyte cells, highlighting the potential risk of skin cancer from melanin.

Allomelanin, also known as miscellaneous color, is produced from the oxidation and polymerization of di-DHN (1,8-dihydroxy naphthalene) or THN (-tetrahydronaphthalene), resulting in colourful shades of allomelanin.

Neuromelanin, a dark polymer color, is found in specific areas of the brain and the adrenal gland in mammals. It is believed to play a significant role in processes such as apoptosis, neurodegeneration, and the development of Parkinson's disease. Its composition includes pheomelanin and eumelanin derived from dopamine and 5-S-cysteinyl dopamine units, linked through rigorous standard analysis methods. Despite numerous studies, the exact function of neuromelanin remains unknown. However, it has been shown to effectively bind substances

like iron, potentially harmful to the brain. The complex nature of neuromelanin is further complicated by its tendency to accumulate in the aging brain.

Pyomelanin, now classified as allomelanin, is a water-soluble color produced during the accumulation and polymerization of homogentisic acid, a by-product of the tyrosine catabolic pathway. This pathway involves the deamination of tyrosine, oxidation, and decarboxylation to produce homogentisic acid, which then undergoes cyclic rearrangement and polymerization to form pheomelanin.

The production of a harmless Pyomelanin pigment by the yeast *Yarrowia lipolytica* was studied. Researchers found that the strain *Yarrowia lipolytica* W29 was able to produce a significant amount (0.5 mg/mL) of extracellular melanin when cultured in a medium containing L-tyrosine. The extracted pigment was found to have antioxidant properties. Similarly, the fungus *Aspergillus fumigatus* is also known to produce Pyomelanin through a similar pathway starting from L-tyrosine. Further proteome analysis revealed that when grown in a medium supplemented with L-tyrosine, the fungus produces enzymes responsible for breaking down L-tyrosine, with homogentisic acid as the primary intermediate. This pathway is of interest as it shares similarities with the L-tyrosine breakdown pathway observed in humans, which leads to the formation of alkaptomelanin. This biochemical connection between *Aspergillus fumigatus* and humans, albeit with different end products, highlights the interconnectedness of natural processes across different organisms (Schmalzer-Ripcke 2020).

There are several benefits of factory melanin in herbal medicine, including its potential as an antitumor agent. Studies have found that factory melanin, similar to herbal melanin (HM), has shown positive effects on colon and skin cancer cell lines, promoting cell death through mitochondrial pathways and regulating important proteins. Additionally, melanin from sources such as *Lachnum YM226* has been found to inhibit liver tumor growth in mouse models. These findings suggest that factory melanin may have a valuable role in herbal medicine by enhancing the sensitivity of cancer cells to treatment and regulating key pathways involved in tumor formation (Guo et al. 2023).

Another benefit of factory melanin is its antioxidant properties, which make it a valuable addition to herbal formulations. Unlike synthetic supplements, factory melanin is unique in its lack of pro-oxidative effects and its ability to work in synergy with other antioxidants like ascorbic acid and glutathione. Recent studies have shown that higher levels of melanin in the blood correspond to increased antioxidant capacity. Furthermore, combining allomelanin with other natural antioxidants in herbal formulations has been found to be safe and devoid of harmful effects often seen with synthetic supplements. Overall, the antioxidant effects of melanin, particularly in herbal medicine, offer various health benefits by addressing conditions, diseases, oxidative stress, and the aging process (Arnao and Hernández-Ruiz 2018).

Melanin is not only produced in the pineal gland but also in other organs known as 'extra-pineal melatonin'. These organs include the gastrointestinal tract, retina, bone marrow, liver, and others (Venegas et al. 2012, Slominski et al. 2008, Tan et al. 1999, Raikhlin et al. 1975). Although extra-pineal melatonin is more abundant than that from the pineal gland, its release into the bloodstream is brief (Bubenik 2002, Huether 1994). Specifically, post-meal responses lead to increases in gut melatonin levels, which have minimal impact on the circadian rhythm due to their short duration. Gastrointestinal melatonin, released during food intake, offers benefits in preventing ulcers, reducing gastric acid secretion, boosting the immune system, improving microcirculation, and promoting tissue regeneration, among other functions (Hardeland and Pandi-Perumal 2005).

Studies have also shown that Phyto melatonin-rich foods have potential as natural sleep enhancers and remedies for jet lag. As melatonin plays a crucial role in sleep quality and

circadian rhythms, consuming foods rich in natural melanin may have a compensatory effect in animals (Aguilera et al. 2016). Limited research suggests that consuming natural foods containing allomelanin can alter circulating levels of melatonin, potentially improving sleep quality and addressing conditions such as insomnia. Age-related sleep disturbances have been linked to reduced nightly peaks of melatonin, and consuming Phyto melatonin-rich foods may offer benefits in managing sleep disorders and promoting post-meal drowsiness (Jan et al. 2000).

The study of natural melanin reveals a range of medicinal properties that make it increasingly important in various fields. This natural polymeric phenolic compound, abundantly found in certain plants, has remarkable antioxidant capabilities, effectively combating oxidative stress and promoting overall health. Additionally, natural melanin has antimicrobial properties, making it a potential candidate for preventing or treating microbial infections. Its hypoglycemic and antihypertensive effects further highlight its therapeutic potential in managing diabetes and hypertension. The compound's interactions with the immune system also emphasize its potential as an immunomodulatory agent, which could aid in the development of immune-boosting nutraceuticals or medicines. As research continues,

Medicinal properties of plant melanin:

Table 2. Medicinal properties of plant melanin

S. No	Plant	Scientific name	Family	Properties of melanin	References
1.	Watermelon	<i>Citrullus lanatus</i>	Cucurbitaceae	Antioxidant properties, potential vigor enhancement for seeds	(Nicolaus et al. 1964, Mavi 2010)
2.	Sunflower	<i>Helianthus annuus</i>	Asteraceae	Resistance to pests, less damage by mole larvae	(Nicolaus et al. 1964)
3.	Buckwheat	<i>Fagopyrum esculentum</i>	Polygonaceae	Not specific	(Zhuravel 2010)
4.	Grapes	<i>Vitis vinifera</i>	Vitaceae	Not specific	(Zherebin and Litvina 1991)
5.	Tomato	<i>Solanum lycopersicum</i>	Solanaceae	Poor germination rate in certain mutants, potential protective functions	(Downie et al. 2003)
6.	Fragrant olive	<i>Osmanthus fragrans</i>	Oleaceae	Not specific	(Wang et al. 2006)
7.	Night jasmine	<i>Cestrum nocturnum</i>	Solanaceae	Not specific	(Kannan and Ganjewala 2009)
8.	Sesame	<i>Sesamum indicum</i>	Pedaliaceae	Not specific	(Panzella et al. 2012)
9.	Ipomoea	<i>Ipomoea tricolor</i>	Convolvulaceae	Not specific	(Park 2012)
10.	Black mustard / Rape	<i>Brassica nigra</i> / <i>Brassica napus</i>	Brassicaceae	Not specific	(Yu 2013)
11.	Chestnut	<i>Castanea</i> spp.	Fagaceae	More cold- and drought-tolerant in certain	(Yao et al. 2012)

				varieties	
12.	Garlic	Allium sativum	Amaryllidaceae	Not specified	(Wang and Rhim 2019)
13.	Oat	Avena sativa	Poaceae	Melanin structure resolved using MALDI-TOF MS, homopolymer from p-coumaric acid	(Varga et al. 2016)
14.	Barley	Hordeum vulgare	Poaceae	Black spike colour under monogenic control, potential protection against pathogens	(Shoeva et al. 2020, Glagoleva et al. 2019)

The properties of plant melanin have been extensively studied and have been found to have various medicinal benefits, leading to its growing importance in different fields. This naturally occurring polymeric phenolic compound, abundant in specific plants, exhibits impressive antioxidant properties that effectively combat oxidative stress and promote overall well-being. Furthermore, plant melanin has been shown to possess antimicrobial properties, indicating its potential in preventing or treating microbial infections. Its hypoglycemic and antihypertensive effects further emphasize its therapeutic properties, suggesting its potential use in managing conditions like diabetes and hypertension. The compound's interactions with the immune system also highlight its potential as an immunomodulator, which could aid in the development of immune-boosting nutraceuticals or medications. Ongoing research has also revealed the anti-inflammatory and anti-tumor activities of plant melanin, offering new possibilities for therapeutic interventions. The diverse medicinal properties of plant melanin make it a subject of great interest for further investigation, with potential for the development of innovative medicines and healthcare solutions.

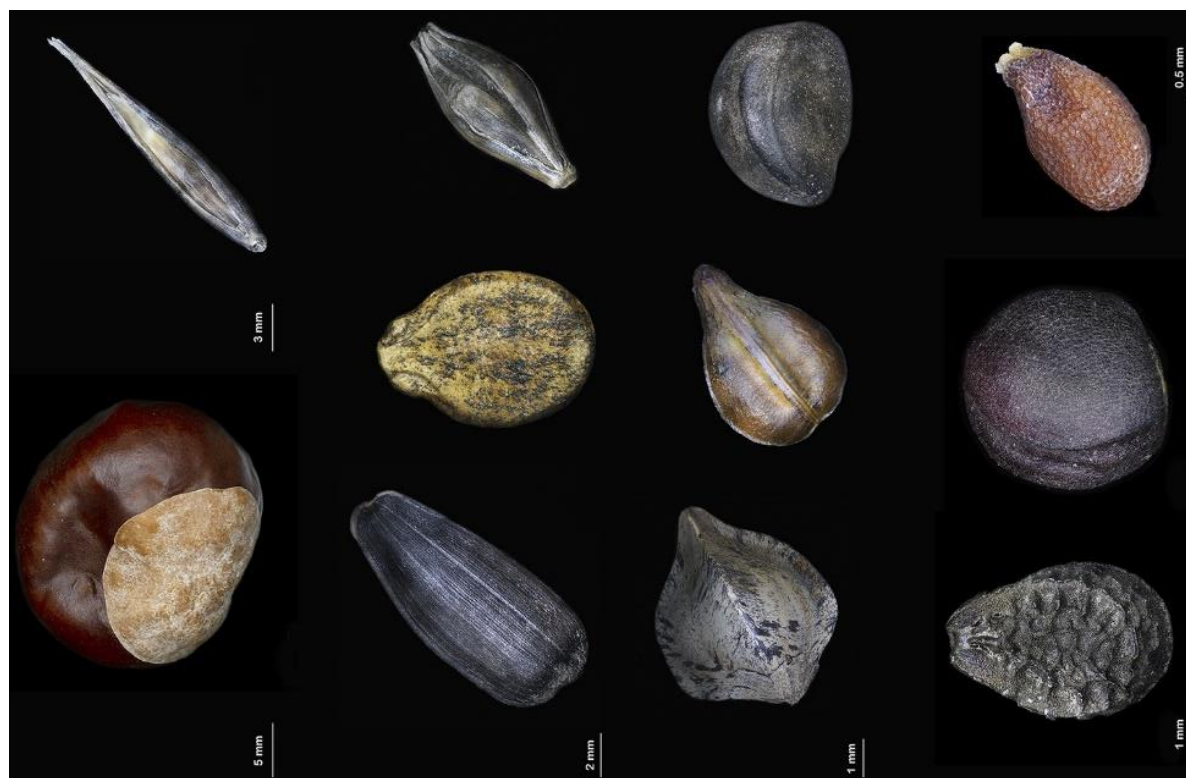


FIGURE 1. *Certain plant species exhibit the accumulation of melanins in their seeds, a phenomenon verified through physicochemical methods. In the illustration, the first row (from left to right) showcases chestnut (*Castanea mollissima*) and oat (*Avena sativa*). Moving to the second row, we have sunflower (*Helianthus annuus*), watermelon (*Citrullus lanatus*), and barley (*Hordeum vulgare*). The third-row features buckwheat (*Fagopyrum esculentum*), grape (*Vitis vinifera*), and ipomoea (*Ipomoea purpurea*). Finally, the fourth row includes sesame (*Sesamum indicum*), rape (*Brassica napus*), and black mustard (*Brassica nigra*) (Glagoleva et al., 2020).*

Potential applications of plant melanin in skincare:

Exploration of each of the potential applications of plant melanin in skincare:

1. **Natural Colorant:** The use of melanin as a non-toxic natural pigment presents an attractive alternative for cosmetic products. Unlike traditional synthetic colorants, which may cause concerns about potential toxicity, allergies, or other negative reactions, melanin offers a safer option. Its incorporation into cosmetic formulas allows for the creation of vivid and desirable shades without the drawbacks commonly associated with artificial colorants. This is particularly beneficial for consumers looking for cleaner and more natural options in the beauty industry. (Liberti 2020, Kalka 2000)
2. **Photoprotective Agent:** The presence of melanin in the skin makes it a valuable component in skincare due to its ability to absorb UV light and act as an antioxidant. Exposure to UV radiation from the sun can cause skin damage, premature aging, and an increased likelihood of skin disorders. Including melanin in cosmetic products may offer a protective barrier against these harmful effects. This not only benefits the user by providing extra UV protection, but also aids in preserving the product and prolonging its shelf life. (Liberti 2020, Kalka 2000)
3. **Anti-Aging Agent:** As we age, our skin undergoes various changes that can lead to the formation of fine lines, wrinkles, and other signs of aging. While there are numerous anti-aging products available in the market, many of them contain synthetic ingredients that may have adverse effects on our health and the environment. However, recent studies have shown that plant melanin may be a safe and effective alternative to synthetic anti-aging agents. In this essay, we will explore the properties and mechanisms of action of plant melanin as an anti-aging agent, its effects on skin health, and its potential applications in the cosmetic and pharmaceutical industry. Plant melanin is a naturally occurring pigment found in various plant species, including mushrooms, seaweed, and some fruits and vegetables. Unlike synthetic melanin, plant melanin is biocompatible and non-toxic, making it a safe alternative for use in anti-aging products. Plant melanin works by reducing oxidative stress and damage to cells, which are major contributors to aging. The opsonizing effect of melanin on the skin implies that it may act as an anti-aging agent. Opsonization is a process that enhances the body's ability to eliminate aging cells, contributing to overall skin health. In the context of skincare, melanin's anti-aging potential suggests that it could help maintain skin vitality, reduce the appearance of fine lines and wrinkles, and support a more youthful complexion. This aligns with the growing demand for anti-aging skincare solutions. (Liberti 2020, Kalka 2000)
4. **Versatile Active Ingredients:** Melanin's classification as a natural nanocomponent with diverse active ingredients underscores its versatility in skincare formulations. The term "nanocomponent" suggests that melanin possesses components at the nanoscale, which can have unique interactions with the skin. These active ingredients may offer various benefits, such as hydration, nourishment, or targeted treatment of

specific skin concerns. The versatility of melanin makes it an attractive option for formulators looking to create skincare products with multifaceted benefits. (Liberti 2020, Kalka 2000)

At the end the potential applications of plant melanin in skincare extend beyond basic coloration. They encompass protection against UV radiation, anti-aging properties, and the versatility of active ingredients. Incorporating melanin into skincare formulations aligns with the preferences of consumers who prioritize natural, effective, and multifunctional beauty products. Researchers and cosmetic developers can explore these aspects further to harness the full potential of plant melanin in the realm of skincare

Plant melanin and its role in protecting plants from UV radiation

Plant melanin, similar to its role in various organisms, plays a vital role in providing inherent protection against harmful UV radiation. This protective mechanism entails the absorption of UV light, preventing potential damage to the skin and lowering the risk of UV-induced disorders (Jablonski and Chaplin 2010).

The absorption of UV light by plant melanin is integral to its photoprotective function. When exposed to UV radiation, melanin molecules within plants absorb the incoming UV photons, transforming this energy into harmless heat. This absorption not only safeguards plant tissues from the adverse effects of UV radiation but also acts as a defense mechanism against oxidative stress.

In the context of human skin, the absorption of UV light by plant melanin can be particularly advantageous. Serving as a natural barrier, it aids in preventing the penetration of UV rays into the deeper layers of the skin, thereby reducing the risk of DNA damage, cell mutation, and the development of UV-induced skin disorders, including skin cancers.

The research by Jablonski and Chaplin in 2010 emphasizes the importance of melanin in this protective process. Their work contributes to the understanding of how melanin, whether in organisms or plants, functions as a biological shield against the detrimental effects of UV radiation. This insight holds significance not only for botanical studies but also for exploring potential applications in human health, such as the formulation of natural sunscreens and other UV-protective products based on plant melanin.

How to extract and utilized plant melanin in research & industry

1. Acid Alkaline method

The research conducted by Chaofeng et al. (2018) details an intricate process for extracting melanin from sunflower seed husks, intending to isolate and purify the pigment for versatile applications. The method involves boiling the sunflower husks in deionized water, followed by cooling and centrifugation to obtain a residue. This residue undergoes homogenization in 1 mol/L NaOH using an ultrasonic processor and is then extracted at 75 °C for 3 hours under reflux in a nitrogen atmosphere. The resulting crude melanin is adjusted to pH 2.0 with 3 mol/L HCl, leading to the precipitation of melanin, followed by centrifugation to collect the pellet.

Further purification steps include hydrolysing the crude melanin with 6 mol/L HCl at 100 °C for 2 hours. The non-hydrolysable melanin obtained is then washed with various solvents and dissolved in 1 mol/L NaOH. The purified melanin is finally obtained through successive centrifugation and lyophilization. To enhance the purification process, the melanin is dissolved in 0.1 mol/L phosphate buffer (pH 8.0) and subjected to chromatography on a Sephadex G-50 column. Fractions are collected, specific fractions are combined, and the resulting mixture is dialyzed, lyophilized, and stored for further use.

Comprehensive physico-chemical properties of the melanin are assessed, including its solubility in various solvents and reactions with oxidizing and reducing agents. Characterization techniques such as UV-Vis's spectrum analysis, FTIR analysis, EPR spectroscopy, elemental composition analysis, and morphology analysis are employed to provide a detailed understanding of the melanin.

The extricated melanin from sunflower seed husks illustrates outstanding antioxidant exercises, as prove by DPPH radical-scavenging, superoxide radical-scavenging, and hydroxyl radical-scavenging tests. Besides, the melanin shows metal particle expulsion movement and anti-radiation properties. These discoveries recommend that sunflower seed husk melanin holds critical potential as an antioxidant, overwhelming metal adsorbent, and UV assurance specialist, advertising promising applications over different businesses, counting nourishment and beauty care products. This comprehensive audit endeavors to examine the extraction and application of plant melanin in both inquire about and industry, explaining its differing applications and potential benefits. The ponder underscores the complex characteristics of melanin determined from sunflower seed husks and its favourable part in assorted mechanical applications

Another one extraction strategy is “Green Nano-particles synthesis” it is additionally called “Green NPs” Nanoscale metallic materials, characterized by grain sizes extending from 5 to 100 nm (Hussain et al. 2016), display particular physical and chemical properties due to the little estimate impact, surface impact, interface impact, and quantum impact. These interesting properties have driven to their broad applications in assorted areas such as science, medication, and designing. Outstandingly, Au nanoparticles (Au NPs) illustrate natural applications, counting protein control and antimicrobial and muscle relaxant exercises (Islam et al. 2015b), whereas Ag NPs hinder the development and exercises of both gram-positive and gram-negative microbes (Ajitha et al., 2014). Fe NPs show antibacterial properties and are viable in cleaning frameworks sullied with natural matter, metals, non-metal particles, and colors (Naseem and Farrukh, 2015; Wei et al., 2017; Fazlzadeh et al. 2017; Weng et al. 2016; Mystrioti et al. 2016). Pd NPs discover applications in color corruption, antimicrobial action, and catalysis (Li et al. 2017; Sharmila et al. 2017; Lebaschi et al. 2017). The blend of nanoscale metals has ended up a central point of investigate, utilizing chemical, physical, and green blend strategies (Wang et al. 2007, Horwat et al. 2011). Whereas physical and chemical strategies posture challenges such as tall vitality utilization, discharge of poisonous chemicals, and complex gear prerequisites, green blend strategies are picking up unmistakable quality due to their eco-friendly approach (Alsammarraie et al. 2018). Green amalgamation utilizes common and environmentally-friendly materials, such as microorganisms or extricates from different plant parts (Devi et al. 2019, Subramaniyam et al. 2015). These materials contain polyphenols and proteins that act as decreasing operators to change over metal particles into lower valence states. For occurrence, green-synthesized Fe₃O₄ nanoparticles have illustrated predominant quality, with a littler molecule measure extend (2–80 nm) compared to those synthesized utilizing damp chemical strategies (87–400 nm) (Gokila et al. 2021). The points of interest of green union, counting its non-toxic, pollution-free, environmentally-friendly, temperate, and economical nature, have been emphasized (Devi et al. 2019, Nasrollahzadeh and Mohammad Sajadi, 2016). Be that as it may, challenges such as constrained accessibility of crude materials, long amalgamation times, and item homogeneity persist. In later a long time, there has been a developing intrigued within the green union of metallic nanoparticles (NPs) due to its eco-friendly nature and potential

applications in different areas. Among these, gold (Au), silver (Ag), palladium (Pd), copper (Cu), and iron (Fe) nanoparticles have gathered critical consideration for their interesting properties and differing applications. 2. Green Union Methods:

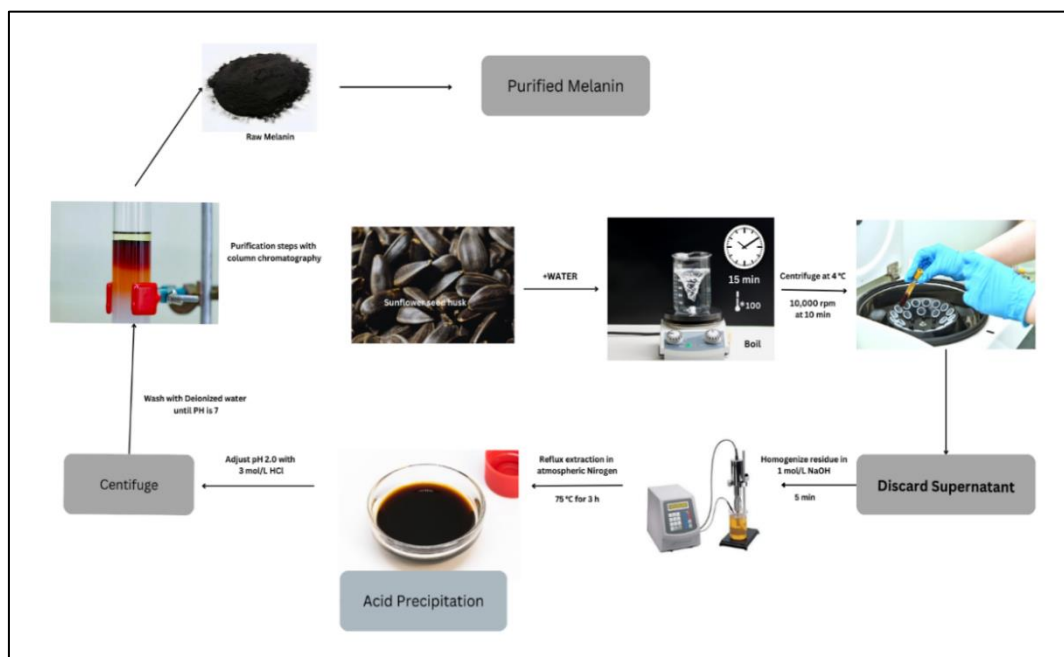


FIGURE 2. Schematic of Acid-alkaline base extraction and purification method of sunflower seed husk.

2.1. Au NPs

The ordinary approach for the green union of Gold Nanoparticles (Au NPs) includes the diminishment of gold particles through plant extricates or microorganisms acting as decreasing operators. Plant extricates are determined by drenching ground plants in solvents such as water or ethanol beneath particular natural conditions. The coming about extricates are at that point combined with an arrangement containing gold particles, driving to the arrangement of Au NPs, apparent when the arrangement turns ruddy (Priya Tharishini et al. 2014, Smitha et al. 2009, Jafarizad et al. 2015, Vijaya Kumar et al. 2019). This strategy has been effectively utilized with extricates from *Cassia auriculata* takes off (Priya Tharishini et al. 2014) and *Cinnamomum zeylanicum* (Smitha et al. 2009), coming about within the diminishment of chloroauric corrosive to Au NPs. The overwhelming shapes of green-synthesized Au NPs are circles, triangles, and hexagons, with varieties depending on the plant extricates utilized. For occurrence, *Pogestemon benghalensis* extricate created circular and triangular Au NPs with distances across extending from 10–50 nm (Paul et al. 2015), whereas *Pelargonium* extricate yielded circular Au NPs with distances across around 10–100 nm (Jafarizad et al. 2015). XRD examination affirmed the face-centered cubic structure of Au NPs with characteristic peaks near 38°, 44°, 64°, and 77° (comparing to the planes of (1 1 1), (2 0 0), (2 2 0), and (3 1 1), individually) (Ranjana et al. 2020, Lee and Stop, 2020). The concentration of chloroauric corrosive altogether impacts the green amalgamation of Au NPs. Thinks about have appeared that the generation of Au NPs at diverse concentrations (extending from 0.51 mM to 4.055 mM) of Au (III) comes about in changing absorbance, demonstrating that Au (III) concentrations underneath 1.53 mM don't Au NPs proficiently (Ahmad et al.

2016). The soundness of Au NPs could be a thought due to their defencelessness to oxidation by discuss. Solidness appraisals utilizing diverse response times and zeta potential estimations uncovered that green-synthesized Au NPs, especially those from plant extricates like Ennab and brown green growth *Cystoseira baccata*, show steadiness and are safe to agglomeration (Aljabali et al. 2018, Gonzalez-Ballesteros et al. 2017). Microorganisms such as yeast *Magnusiomyces ingens* LH-F1, HS-11, and mesophilic filamentous parasites have also been utilized for the green amalgamation of Au NPs (Zhang et al. 2016, Sowani et al. 2016, Vágó et al. 2016). Analysts are investigating the applications of green-synthesized Au NPs, with a centre on their catalytic and restorative employments. For case, Quercetin-synthesized Au NPs illustrate catalytic action within the debasement of 4-nitrophenol and methyl orange, as well as the discovery of alkali (Kumari and Meena, 2020). Also, Au NPs discover broad applications within the therapeutic industry, serving as carriers for bioactive particles like anticancer operators, upgrading adequacy in clinical pharmaceutical (Lee et al. 2020).

2.2. Ag NPs

The green amalgamation of Silver Nanoparticles (Ag NPs) ordinarily includes blending silver nitrate arrangement with lessening substances extricated from plants. Different plant extricates, such as *Tephrosia purpurea* leaf powder, coconut coir extricates, and grass squanders, have been utilized to synthesize Ag NPs with particular shapes and sizes (Ajitha et al. 2014, Roopan et al. 2013, Khatami et al. 2018). Ag NPs have been investigated for their antibacterial properties, but considers demonstrate potential impacts on plant development, with noteworthy hindrance watched in a few cases (Ruttkay-Nedecky et al. 2019, Sehnal et al. 2019). Variables impacting the green blend of Ag NPs incorporate the plant extricate, pH, and temperature. Diverse parts of the same plant may deliver Ag NPs with shifting impacts, as watched in *Sesuvium portulacastrum* L's callus and leaf extricates (Nabikhan et al. 2010). pH and temperature varieties too affect the measure and dissemination of Ag NPs (Jebakumar Immanuel Edison and Sethuraman, 2013, Ping et al., 2018). Steadiness testing includes deciding the ideal response time to anticipate accumulation and precipitation (Venkatesham et al. 2014). Ag NPs have been connected in different areas, such as catalysis, restorative medicines, and electronic gadgets (Ahmed et al. 2016, Ahmed et al. 2017, Khatami et al. 2019).

2.3. Pd NPs

Palladium Nanoparticles (Pd NPs) synthesized through green strategies show interesting properties, counting antibacterial and catalytic exercises. The green union of Pd NPs ordinarily includes the utilize of plant extricates, highlighting the flexibility of these eco-friendly approaches (Patel et al. 2015, Chandra and Kulshreshtha, 2017). Pd NPs synthesized from plant extricates appear potential applications in pharmaceutical, acting as viable antibacterial specialists against both Gram-negative and Gram-positive microbes (Iravani et al. 2014). The catalytic properties of green-synthesized Pd NPs have been investigated in different chemical responses, illustrating their potential as catalysts for Suzuki coupling responses, decrease of nitro compounds, and hydrogenation responses (Ghosh et al. 2012, Rostamizadeh et al. 2014).

2.4. Cu NPs

Copper Nanoparticles (Cu NPs) synthesized through green strategies are picking up consideration due to their assorted applications, extending from antibacterial operators to catalysts in chemical responses. The green blend of Cu NPs can be accomplished utilizing microbial or plant extricates (Gopinath et al. 2015, Yallappa et al. 2015).

Microbial union includes the utilize of microbes or organisms as decreasing operators, whereas plant extricates serve as decreasing and capping specialists, contributing to the stabilization of Cu NPs (Mude and Ingle, 2018, Mude et al. 2019). Variables affecting the measure and solidness of green-synthesized Cu NPs incorporate the concentration of plant extricate, capping specialists, and surfactants (Gopinath et al. 2015, Siddiqi et al. 2018). The green blend of Cu NPs is considered cost-effective and naturally inviting compared to routine strategies including chemical decreasing operators (Mude et al. 2019).

2.5. Fe NPs,

NZVI, and Press Oxide NPs Press Nanoparticles (Fe NPs), Nanoscale Zero-Valent Press (NZVI), and Press Oxide Nanoparticles are synthesized utilizing different plant extricates, contributing to their soundness and adequacy. Green amalgamation strategies have been utilized for the planning of Fe NPs with differing applications, counting wastewater treatment, soil remediation, and antimicrobial considers (Nanda and Saravanan, 2009, Rao et al. 2019).

Plant extracts, such as those from *Tridax procumbens* and *Annona squamosa*, have been utilized for the green synthesis of Fe NPs with potential applications in removing heavy metals from contaminated water (Singaravelu et al. 2007, Pattanayak and Nayak, 2013).

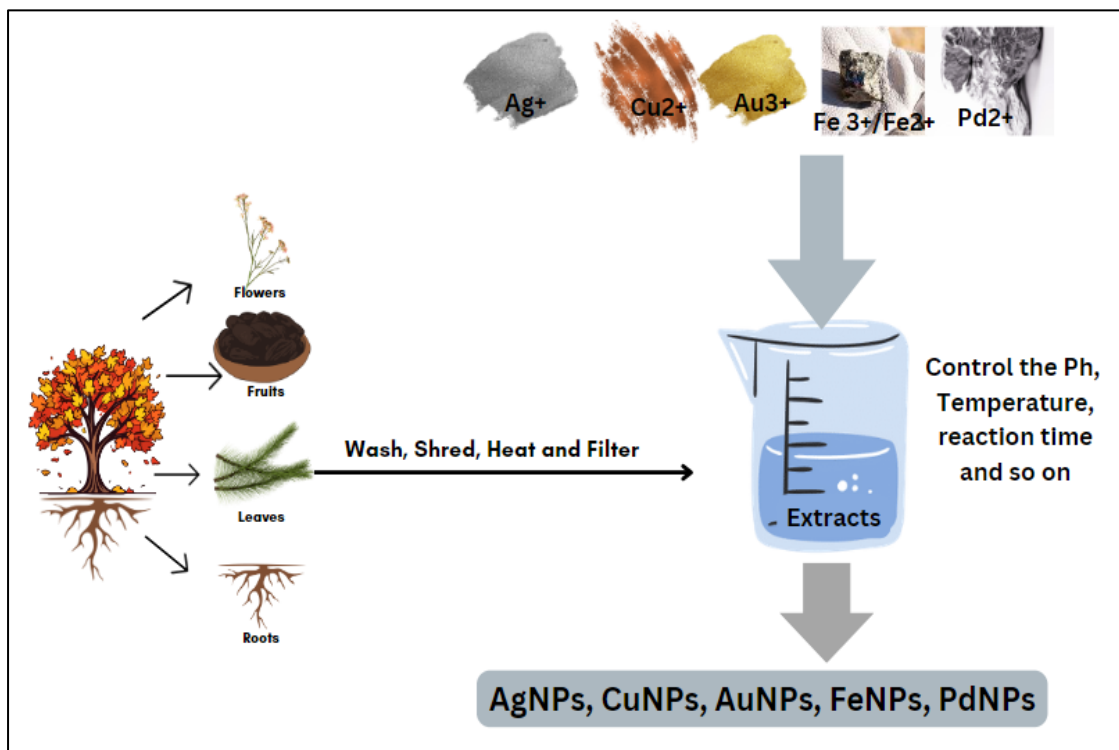


FIGURE 3. Synthesis of Nanoscale Metals Utilizing Extracts from Various Plant Parts

Similarly, NZVI, with its unique properties, has been synthesized using plant extracts like *Aloe vera* and *Azadirachta indica* (Al-Gheethi et al. 2016, Ali et al. 2016). The green synthesis of Iron Oxide Nanoparticles has been achieved using plant extracts like *Neem (Azadirachta indica)* and *Aloe vera*, showing potential applications in biomedical fields (Kumar et al. 2011, Rajakumar et al. 2018)

Challenges and Future Prospects of Plant Melanin Research

Challenges of Plant Melanin Research:

1. **Complex Polymeric Nature:** Plant melanin presents a significant challenge due to its complex polymeric nature. This natural pigment exists as a heterogeneous group with diverse origins, colors, compositions, and functions. Unravelling the intricate structure of plant melanin requires advanced biochemical and molecular genetic studies to comprehend its complexities fully.
2. **Localization in Seed Envelopes:** The specific localization of plant melanin in seed envelopes poses a challenge, especially as other compounds with similar colors, such as proanthocyanidins, can coexist in these environments. This coexistence complicates the isolation and study of plant melanin, necessitating meticulous approaches for accurate analysis.
3. **Limited Studies in Plants:** Unlike animals and microorganisms, plant melanin has received relatively less attention in biochemical and molecular genetic research. This knowledge gap underscores the necessity for more comprehensive research efforts to understand melanin formation in plants.
4. **Need for Phytochemical Verification:** Verifying the melanic nature of plant pigment requires specific phytochemical and herbal methods. Developing reliable and standardized verification methods is crucial to ensure the accuracy and validity of research outcomes.

Future Prospects of Plant Melanin Research:

1. **Biosynthesis Pathways in Plants:** Future research should delve deeper into the biosynthesis pathways of melanin in plants, aiming to understand the genetic and biochemical aspects of melanin formation comprehensively.
2. **Functional Roles in Plants:** Investigating the functional roles of melanin in plants beyond coloration is crucial. Understanding how melanin contributes to plant resilience, protection, and response to environmental factors presents a promising avenue for future research.
3. **Applications in Agriculture:** Exploring the potential applications of plant melanin in agriculture, such as enhancing resistance to pests or environmental stressors, could lead to sustainable and innovative farming practices.
4. **Standardization of Extraction Methods:** Establishing standardized methods for the extraction of plant melanin is essential for consistency in research. Optimizing extraction procedures for different plant sources ensures reliable and comparable results across studies.
5. **Integration with Nanotechnology:** The integration of plant melanin with nanotechnology presents exciting possibilities for applications in medicine, cosmetics, and other industries. Exploring how plant melanin can be utilized in conjunction with nanomaterials opens new avenues for research.
6. **Health and Medicinal Applications:** Further exploration of the health benefits and medicinal properties of plant melanin, including its antioxidant and anti-tumor potential, holds significant promise and may lead to the development of novel therapeutic interventions.
7. **Environmental and Sustainable Practices:** Research focusing on the environmental and sustainable aspects of plant melanin is essential, aligning with global efforts toward green and sustainable practices. Understanding how plant melanin contributes to environmental protection and conservation is an important area of study.

Title	Methodology	Results	References
Extraction and Purification of melanin from various cells and tissues	This involves extracting melanin from various sources like animals, plants, and microbes using different techniques such as acid/base extraction, enzymatic extraction, ultrasound-assisted alkaline hydrolysis, and organic solvent extraction. Challenges related to current methods are also explored. Specific methods include alkali-acid extraction, acid/base extraction, enzymatic extraction, ultrasound-assisted alkaline hydrolysis, boiling in water, and organic solvent extraction. The study aims to overcome existing limitations and advance melanin extraction for diverse applications.	Indicate that melanins, crucial for photoprotection and survival, are synthesized by living organisms. Extraction procedures differ among cells and tissues, with bacteria presenting a simpler isolation process. However, the corrosive nature of acids and alkalis used in extraction makes the process economically challenging. Therefore, alternative techniques like TBAOH extraction warrant further exploration to improve feasibility and efficiency.	<i>Noble K Kurian (2022)</i>
Melatonin in higher plants: occurrence and possible functions.	Techniques such as solid-phase extraction, high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS/MS) were employed. Additionally, immunoaffinity chromatography and radioimmunoassay (RIA) were utilized for sample purification and melatonin level determination. The study aimed to accurately assess melatonin presence and levels in plants, providing valuable insights for further research and applications.	Melatonin in plants has diverse potential functions such as delaying flower induction, acting as an antioxidant, exhibiting auxin-like effects, and signaling plant interactions with herbivores and pests. However, further research is necessary to comprehensively grasp its roles and impacts in plants.	<i>Jan Kolar & Ivana Machackova (2005)</i>

<p>Effect of Ultra-Fine Grinding on the Structure of Plant Raw Materials and the Kinetics of Melanin Extraction</p>	<p>Involved mechanical treatment of plant raw materials, X-ray diffraction (XRD) analysis for determining crystallinity, particle size measurement using an optical analyzer, spectrophotometric analysis, and fitting extraction kinetics using various models.</p>	<p>Study examined the impact of mechanical treatment on polyphenol extraction kinetics from two raw material types. It found that the Baker equation and Lonsdale model offered superior descriptions of the extraction process compared to the first-order equation. Furthermore, extraction kinetics of melanin from Ganoderma applanatum and buckwheat husk were effectively fitted by models rooted in Fickian diffusion, with consideration for the particle shape-factor.</p>	<p><i>Lomovskiy, I et al (2021)</i></p>
<p>Risk of Migraine in Europeans with Low Melanin Levels -A Population Based Case-Control Study</p>	<p>Comprised measuring skin pigmentation parameters on the inner part of both arms in adults with migraine and a control group, utilizing a DSM II Cortex Technology dermo spectrophotometer. Parameters included the melanin index, erythema index, and analysis in the CIE Lab color space and RGB color model. Statistical analysis was conducted using the STATISTICA 13.0 program.</p>	<p>This study revealed that individuals with a low melanin index faced a more than threefold increased risk of migraine. Additionally, fair skin, characterized by lighter pigmentation, was linked to higher migraine prevalence. Notably, this study represents the first investigation into the correlation between skin pigmentation and migraine risk.</p>	<p><i>Kobus, M, et al 2022</i></p>
<p>Physicochemical properties and the extraction process of natural melanin from Auricularia polytricha</p>	<p>The methodology systematically analysed A. polytricha melanin utilizing SEM, UV-Vis's spectroscopy, IR spectroscopy, EPR spectroscopy, and elemental analysis. It explored solubility, stability, and antioxidant activities, with extraction employing cellulase-ultrasonic synergistic technology.</p>	<p>The study successfully isolated and characterized melanin from A. polytricha using multiple analytical techniques. The melanin exhibited favourable solubility in alkaline solutions, low solubility in distilled water and organic solvents, stability against most metal ions, and demonstrated antioxidant activity.</p>	<p><i>Junsheng Fu, (2018)</i></p>

<p>Natural Melanin: Current Trends, and Future Approaches, with Especial Reference to Microbial Source</p>	<p>The methodology involves a literature review on microbial melanin, structured and Various analytical techniques such as MALDI analysis, ESCA, py-GC-MS, LC-MS, EPR/ESR, X-ray diffraction, and NMR spectroscopy are utilized.</p>	<p>Answer not found</p>	<p><i>El-Naggar et al 2022</i></p>
<p>Melanins: Skin Pigments and Much More-Types, Structural Models, Biological Functions, and Formation Routes</p>	<p>It involves techniques such as hot oxidation with permanganate or hydrogen peroxide in alkaline media, the role of tyrosinase in melanin biosynthesis, utilization of tyrosinase from M. Mediterranean, presence of laccase and phenol oxidases in fungal species, and tyrosinases with high affinity for L-tyrosine.</p>	<p>Melanin, a widespread and enduring pigment, is present in diverse organisms. Its formation entails enzymatically-controlled phases and uncontrolled polymerization of oxidized intermediates. The biochemistry of melanin is actively studied in dermatology, biomedicine, cosmetics, microbiology, and fruit technology fields.</p>	<p><i>F. Solano, 2014</i></p>
<p>Substantia nigra neuromelanin: structure, synthesis, and molecular behaviour</p>	<p>The methodology comprised reviewing studies on the molecular aspects of neuromelanin, conducting electron paramagnetic resonance (EPR) and metal analysis studies, degradation analyses using potassium permanganate and hydriodic acid hydrolysis, and x-ray diffraction studies. Additionally, degradation analyses using potassium permanganate and hydriodic acid hydrolysis, induction of neuromelanin synthesis with L-dopa, iron chelation with desferrioxamine, and neuropathological investigations were conducted.</p>	<p>Neuromelanin is a genuine melanin with chelating abilities for iron and interacts with various compounds, potentially playing a protective role. It accumulates with age, interacts with toxic compounds, and can influence their toxicity. Neuromelanin can sequester iron ions to reduce oxidative stress but may become cytotoxic under certain conditions.</p>	<p><i>Zecca L, et al 2001</i></p>

<p>Preliminary Characterization of Melanin Isolated from Fruits and Seeds of <i>Nyctanthes arbor-tristis</i></p>	<p>Isolating and purifying melanin from <i>Nyctanthes arbor-tristis</i> fruits and seeds via alkaline extraction, acid hydrolysis, and organic solvents. The isolated melanins underwent characterization using UV-visible and IR spectroscopy, followed by stability assessment through temperature variations and exposure to oxidants and metal ions. Additionally, techniques such as alkaline extraction, acid hydrolysis, organic solvent purification, spectrophotometry (including infrared spectroscopy), heat stability testing, and evaluation of the effects of oxidants and metal ions were employed.</p>	<p>Melanin from <i>Nyctanthes arbor-tristis</i> fruits and seeds was isolated and purified using specific methods. The study found that fruit melanin exhibited greater stability at 50 °C, whereas seed melanin was more stable at 25 °C. However, both types of melanin were affected by oxidants and metal ions.</p>	<p><i>Kannan, P., & Ganjewala, D. (2009)</i></p>
<p>Physicochemical Properties and Biological Activities of Melanin Extracted from Sunflower Testae</p>	<p>The study involved extracting and purifying melanin from sunflower testae, followed by a comprehensive assessment of its physicochemical properties, spectroscopic characterization, elemental composition, morphology, and bioactivity. Various techniques such as UV-vis spectroscopy, FT-IR, EPR spectroscopy, SEM, and elemental analysis were employed. Additionally, bioactivity assays including DPPH radical-scavenging, superoxide radical scavenging, hydroxyl radical scavenging, reducing power determination, metal ion absorption,</p>	<p>Melanin extracted from sunflower testae exhibited antioxidant potential across multiple assays, suggesting its viability as a natural antioxidant with potential applications across diverse industries.</p>	<p><i>Chao feng Li, et al (2018).</i></p>

	and anti-radiation assays were conducted.		
Melanin Pigment in Plants: Current Knowledge and Future Perspectives	Data collection focused on plant melanin, emphasizing its functions, localization, and molecular-genetic control. The emphasis was on confirming the melanin nature of the pigment through physicochemical methods. Chemical tests and spectroscopic techniques were utilized to verify melanin presence in seeds from various plant species. Techniques included alkaline extraction, precipitation in acid conditions, UV-Vis's spectroscopy, FT-IR spectroscopy, NMR analysis, EPR spectroscopy, MALDI-TOF MS, and various chemical and physicochemical methods.	Plant melanin primarily contributes to enzymatic browning in wounded tissues. The genetic process of melanin synthesis in plants is intricately regulated and involves multiple genes, with a potential pivotal role for genes encoding PPOs. The paper offers insights into the current understanding of plant melanin and explores potential future research avenues.	<i>Glagoleva AY, et al (2020)</i>
Physicochemical properties and biological activities of melanins from the black-edible fruits Vitex mollis and Randia echinocarpa	Involved extracting, purifying, and characterizing melanins from Vitex mollis and Randia echinocarpa fruits, followed by assessing their antioxidant and immunomodulatory activities. Various analytical techniques such as solubility testing, redox stability evaluation, metal ion interaction assays, phenolics quantitation, thermogravimetric analysis, elemental analysis, UV-vis spectroscopy, FTIR-ATR spectroscopy, and NMR spectroscopy were employed.	Melanins extracted from Vitex mollis and Randia echinocarpa, derived from tyrosine, demonstrate potential as photoprotective agents and for the prevention/treatment of chronic degenerative diseases. Vitex mollis melanins exhibited superior antioxidant activity compared to Randia echinocarpa melanins. Both melanins remained stable under artificial light but were bleached by sunlight, with Vitex mollis melanins being more susceptible. They were stable in reducing agents but were bleached by oxidants such as KMnO ₄ and K ₂ Cr ₂ O ₇ .	<i>Montes-Avila, J., et al (2018).</i>

<p>Isolation, characterization and anti-oxidant potential of melanin pigment from Trichomerium bhatii NFCCI 4305</p>	<p>The methodology described extracting and purifying melanin pigment from Trichomerium bhatii using the alkali-acid method. Subsequently, spectral analysis is conducted through UV spectroscopy and FTIR spectroscopy, while elemental analysis is performed using an Elemental analyzer. Physico-chemical tests, UV spectroscopic analysis, FTIR spectra analysis, and elemental analysis are key components of the methodology.</p>	<p>Extracting and purifying melanin pigment from Trichomerium bhatii using the alkali-acid method. Spectral analysis was conducted through UV spectroscopy and FTIR spectroscopy, along with elemental analysis using an Elemental analyzer. Physico-chemical tests, UV spectroscopic analysis, FTIR spectra analysis, and elemental analysis were key components of the methodology. The melanin exhibited robust antioxidant activity, categorized as DOPA melanin based on elemental analysis, and displayed characteristics similar to synthetic DOPA melanin, suggesting potential applications in derma-cosmetic industries.</p>	<p><i>Malika Suthar, Sanjay K. Singh. (2023)</i></p>
<p>Advances in green synthesis of nanoparticles</p>	<p>Green synthesis methods utilizing plant metabolites and natural substances to produce nanoparticles, with a focus on reducing toxicity compared to traditional methods. Techniques utilized include UV-vis absorption spectroscopy, X-ray diffraction (XRD), Fourier transmission infrared (FTIR) spectroscopy, dynamic light scattering (DLS), Energy dispersive X-ray examination (EDAX), scanning electron microscopy (SEM), and transmission electron microscopy (TEM).</p>	<p>The significance of green synthesis methods utilizing plant metabolites for nanoparticle production, highlighting their potential to reduce toxicity and their applications in various fields.</p>	<p><i>Aman Gour & Narendra Kumar Jain (2019)</i></p>
<p>Preparation and</p>	<p>Extraction of pure melanin from Nigella</p>	<p>The main findings of the study include the</p>	<p><i>Ghada Khouqeer,</i></p>

<p>characterization of natural melanin and its nanocomposite formed by copper doping</p>	<p>Sativa seeds and the formation of nano-composite melanin by doping it with copper. Characterization involved various analytical techniques to investigate properties such as magnetic behavior. Techniques included TGA, TEM, XRD, VSM, UV-Vis's spectroscopy, RAMAN spectroscopy, FTIR, NMR, and EPR spectroscopy.</p>	<p>non-crystalline nature of Mel and Cu-Mel, as well as the enhancement of magnetic properties through copper doping.</p>	<p><i>et al (2022).</i></p>
<p>The Potential of Phytomelatonin as a Nutraceutical</p>	<p>The methodology comprised extracting pure melanin from Nigella Sativa seeds, followed by doping with copper to form Cu-Mel. Characterization was conducted using various analytical techniques including TGA, TEM, XRD, VSM, UV-Vis's spectroscopy, RAMAN spectroscopy, FTIR, NMR, and EPR spectroscopy. The extraction process involved dissolving seed coats in NaOH and subsequent purification steps. Cu-Mel preparation encompassed multiple chemical procedures, and experiments were conducted using specialized equipment for each analytical technique.</p>	<p>Phytomelatonin, with its natural origin, offers diverse protective effects in plants, presenting promising opportunities. It plays numerous roles in modulating circadian rhythms, mood, sleep, and possesses antioxidant properties. Melatonin exhibits potential as a treatment for cancer and is linked to various health benefits.</p>	<p><i>Marino B. et al (2018).</i></p>
<p>Isolation and Characterization of Allomelanin from Pathogenic Black Knot Fungus Sustainable Source of Melanin</p>	<p>Extracting melanin from black knots using the acid-base extraction method. Subsequently, chemical characterization is performed using various spectroscopic techniques, including</p>	<p>Melanin extracted from black knots exhibits irregular morphology and broadband UV absorption typical of other melanins, confirming it as allomelanin and suggesting its potential use for UV</p>	<p><i>Saranshu Singla, (2021)</i></p>

	SEM, TEM, XPS, FTIR, ss-NMR, and UV-Vis spectrophotometry for morphology analysis and UV-absorption analysis.	protection in various applications.	
Melanin is a plenteous bioactive phenolic compound in date fruits (Phoenix dactylifera L.)	Involved characterizing melanin extracted from date fruits using UV-visible and FTIR spectra, XRD, XPS, TGA, SEM, EDS, HPLC, ATR-FTIR, light microscopy, elemental mapping, and nitrogen sorption analysis.	Date palm fruits contain high levels of allomelanin with various beneficial effects, emphasizing the importance of investigating its dietary intake and nutritional impact. The study provides evidence of the nature of melanin in date fruits through various analytical methods.	<i>Alam, M.Z., et al. (2022).</i>
Characterization of the physicochemical properties, antioxidant activity, and antiproliferative activity of natural melanin from S. reiliana	The methodology focused on examining the physicochemical properties and stability of L-25 melanin, assessing its solubility, response to environmental conditions, and antioxidant activity using the DPPH free radical scavenging method. Techniques employed included scanning electron microscopy (SEM), dynamic light scattering (UPLC-QTOF-MS), and Fourier-transform infrared spectroscopy.	Melanin extracted from S. reiliana demonstrates potent antioxidant activity, exhibiting strong DPPH free radical scavenging ability. Additionally, it can protect damaged HepG2 cells by reducing oxidative stress markers. Furthermore, it displays moderate antioxidant activity in melanoma cells.	<i>Fu, X., et al (2022).</i>
Melanin-like nanoparticles: advances in surface modification and tumour photothermal therapy	Use of synthetic melanin nanoparticles with reactive groups for applications like photovoltaics and photoacoustic techniques. It underscores the importance of multidisciplinary collaboration to facilitate the clinical translation and commercial breakthroughs of these nanoparticles (MNPs).	Melanin preparation methods, polymerization mechanisms, and physicochemical properties essential for designing melanin nanoparticles (MNPs). Additionally, it highlights recent advancements in polydopamine (PDA) melanin within oncology. Moreover, it discusses the diverse applications of MNPs across various fields.	<i>Tian, L., (2022).</i>

<p>Recent Advances and Progress on Melanin: From Source to Application</p>	<p>Analytical techniques included macromolecular structure analysis, investigation of the polymerization process, resistance testing against acids, light, and reducing agents, as well as solubility and heat resistance testing.</p>	<p>Melanin, a widely available biological pigment, possesses unique properties and excellent biocompatibility, rendering it valuable across diverse fields including biomedicine, agriculture, and the food industry. It showcases robust free radical scavenging activity against various radicals such as DPPH, superoxide, and hydroxyl radicals, suggesting its potential as an antioxidant. Additionally, melanin exhibits promising antitumor activity, including light-absorbing properties that may offer protection against skin cancer induced by factors like ultraviolet radiation.</p>	<p><i>Guo, L.; 2023</i></p>
<p>From Extraction to Advanced Analytical Methods: The Challenges of Melanin Analysis</p>	<p>Techniques covered include matrix-assisted laser desorption/ionization mass-spectrometry, pyrolysis gas chromatography, Fourier-transform infrared spectroscopy, proton and carbon nuclear magnetic resonance, thermogravimetric analysis, scanning electron microscopy, transmission electron microscopy, atomic force microscope, liquid chromatography, multiple reaction monitoring, high-performance liquid chromatography coupled with mass spectrometry detection, reversed phase liquid chromatography, hydroiodic acid hydrolysis, alkaline H₂O₂ oxidation-ion pairing agent, sodium octane sulfonate, and HILIC.</p>	<p>Include the diverse properties and functions of melanins, justifying research interest, and a detailed overview of methods for melanin analysis.</p>	<p><i>Pralea I-E, et al. 2019</i></p>

<p>Extraction, physicochemical properties, and antioxidant activity of natural melanin from <i>Auricularia heimuer</i> fermentation</p>	<p>Use single-factor experiments, Box-Behnken design (BBD), and response surface methodology (RSM) to investigate specific parameters' effects on AHM extraction yield. Various analytical techniques such as ultraviolet-visible spectrum (UV-Vis), Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscope (SEM), and high-performance liquid chromatography (HPLC) were utilized for analysis. Additionally, the solubility, stability, and antioxidant activities of AHM were measured.</p>	<p>The extraction conditions for AHM, demonstrated its absorption characteristics at 210 nm, and highlighted its solubility properties and antioxidant activities.</p>	<p><i>Ma Y, et al (2023)</i></p>
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Conclusion

In conclusion, this thorough review provides a deep exploration of plant melanin, elucidating its synthesis, phytochemical composition, and significance in herbal medicine. It categorizes different types of melanin, highlighting their distinct characteristics and functions. Emphasis is placed on the medicinal properties, extraction procedures, and versatile applications of plant melanin, from skincare to potential agricultural uses. The review acknowledges challenges in understanding its complex nature and localization, paving the way for future research on biosynthesis pathways, functional roles, and environmental sustainability for a comprehensive grasp of plant melanin.

Author Contributions

Dr. Neha Pal wrote the original draft of the manuscript. **Prof. Anwar Shahzad** contributed to the conceptualization, provided supervision, guidance, and made critical revisions for intellectual content. Both authors, **Dr. Neha Pal** and **Prof. Anwar Shahzad**, actively participated in the literature review, drafting, and thorough review and editing of the manuscript.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this review article. They have no financial or personal relationships that could potentially bias or influence the work presented herein.

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