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Pigment extraction from mulberry: A review

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Abstract
Mulberry plants (*Morus* spp.) represent a promising source of natural pigments, particularly anthocyanins and carotenoids, with significant potential in the food, textile, cosmetic, and pharmaceutical industries. This review consolidates current knowledge on the extraction, purification, characterization, and applications of pigments from mulberry leaves and fruits. Conventional solvent extraction, employing polar solvents such as ethanol, methanol, and acetone under acidic conditions, remains the most widely used method, while emerging techniques like ultrasound-assisted and microwave-assisted extraction offer enhanced efficiency and reduced processing time. Optimized extraction parameters—including solvent type, temperature, pH, and material-to-liquor ratio—are crucial for maximizing pigment yield and stability. Mulberry leaves are rich in lutein and β-carotene, whereas fruits contain abundant anthocyanins, primarily cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside, which also exhibit potent antioxidant and therapeutic properties. Beyond their coloring potential, mulberry pigments demonstrate bioactivities such as anti-inflammatory, hepatoprotective, and anticancer effects. The growing consumer demand for sustainable, safe, and functional colorants underscores the relevance of advancing eco-friendly extraction technologies and expanding commercial applications of mulberry-derived pigments.

Keywords: Mulberry, *Morus* spp., natural pigments, anthocyanins

Introduction
The various colors seen in plants, animals, and microbes are caused by pigments, which are found throughout the biological world (Siddharthan *et al.*, 2020) [17]. Because of their potential uses in a variety of industries, such as food, cosmetics, textiles, and pharmaceuticals, the extraction of these pigments from natural sources has attracted a lot of attention (Juric *et al.*, 2020) [10]. A promising source of natural pigments, especially anthocyanins, which give its fruits their vivid colors, is the genus of flowering plants known as mulberries, which belong to the Moraceae family (Suhaimi *et al.*, 2021) [18]. As primary or secondary metabolites, pigment production in microorganisms is essential to their survival and plays a key role in photoreactive mechanisms that protect against oxidative stress and reduce DNA damage from UV radiation (Ghosh, 2025) [8]. The process of pigment extraction from mulberry plants involves a multifaceted approach, encompassing various techniques aimed at isolating and purifying the desired compounds while preserving their structural integrity and bioactivity. The recovery of anthocyanins and phenolic compounds from plant materials is commonly achieved through solid-liquid extraction utilizing polar solvents such as methanol, ethanol, and acetone under acidic conditions (Suhaimi *et al.*, 2021) [18]. Natural sources, chemical characteristics, stabilization procedures, and applications in actual food systems are some of the major concerns surrounding the use of these natural colorants in foods. They cover a variety of pigment groups, many of which have strong biological potential. Major regulatory bodies have approved the use of natural pigments in foods, which provide a variety of colors and intensities. However, their higher cost and less stability in comparison to synthetic alternatives can occasionally impede their adoption (Juric *et al.*, 2020) [10].
The choice of extraction method is contingent upon several factors, including the type of pigment being extracted, the plant matrix, and the desired purity and yield. Solvent extraction, a conventional technique widely employed at the laboratory scale, involves the use of organic solvents to selectively dissolve and separate target compounds from insoluble solids through absorption and release of bioactive components (Kattil *et al.*, 2024) [12]. The efficiency of solvent

extraction is influenced by several parameters, including solvent type, temperature, extraction time, and solid-to-solvent ratio. The selection of a suitable nontoxic extraction solvent is critical for the safe recovery of pigments intended for use as food colorants (Daud *et al*., 2021) [7]. Optimization of anthocyanin extraction involves careful control of factors like solvent type to maximize extraction rates (Tan *et al*., 2022) [19].

Mulberry plants, which are members of the Morus genus, are a valuable but underutilized resource because of their varied nutritional makeup, phytochemical richness, and possible pharmacological uses (Kattil *et al*., 2024) [12]. Like other berries, mulberry fruits are syncarps made up of clusters of tiny fruits called drupes that undergo color, texture, and biochemical changes as they ripen following pollination (Kattil *et al*., 2024) [12]. The fact that these plants are found in temperate and subtropical climates in Asia, Africa, Europe, and the Americas highlights their accessibility and potential for pigment extraction projects (Chetia *et al*., 2023) [6].The shift towards natural pigments has accelerated, driven by heightened awareness of the safety and potential toxicity associated with synthetic counterparts, thereby creating a compelling impetus for exploration of plant-based alternatives such as mulberry (Bakhsh *et al*., 2023) [3]. The extraction of pigments from mulberry plants is a multifaceted process influenced by several critical parameters, including the choice of extraction solvent, the optimization of extraction methodologies, and precise control over environmental conditions such as temperature, pH, and light exposure. Solvent extraction is a common and conventional technique for extracting pigments from plant materials that relies on dissolving the desired compounds in a suitable solvent (Kalra *et al*., 2020) [11]. Polar solvents, including ethanol and acetone, have demonstrated efficacy in extracting anthocyanins from mulberry fruits, underscoring their role in solubilizing these pigments (Suhaimi *et al*., 2021) [18]. Novel technologies, including ultrasound-assisted extraction and microwave-assisted extraction, have emerged as promising alternatives to conventional solvent extraction methods, as they offer the advantages of reduced extraction time, lower solvent consumption, and enhanced extraction efficiency (Masyita *et al*., 2025) [15]. These advanced techniques leverage the principles of sonication and microwave irradiation, respectively, to disrupt plant cell walls and facilitate the release of pigments into the extraction solvent. Furthermore, the optimization of extraction parameters, such as solvent composition, extraction time, temperature, and pH, is crucial for maximizing pigment yield and purity. Different cultivars of mulberry exhibit variations in anthocyanin profiles and antioxidant activity, suggesting the importance of cultivar selection in pigment extraction strategies (Kim & Lee, 2020) [13].

Pigment Extraction from Mulberry

From sample preparation to the ultimate pigment isolation and purification, the process of extracting pigments from mulberry plants usually entails multiple steps. The particular process can change based on the desired pigment type and whether the leaves or fruits of the mulberry plant are being used. Gather fresh mulberry leaves, usually from certain spots (such as third and fourth from the top). Use distilled water to thoroughly wash the gathered leaves in order to get rid of any dirt or contaminants. To maximize the surface area for extraction, chop them into smaller pieces, such as those that are about 1 cm in size (Chetia *et al*., 2023) [6]. Fresh mulberry fruits can be processed into juice (Liu *et al*., 2004) [14] or consumed raw (Qin *et al*., 2010) [16]. Mulberry pomace, a byproduct of juice and wine making, can also be utilized (Zhang *et al*., 2011) [21].

Various methods are employed for extracting pigments, often involving different solvents and conditions.

Pigments from Mulberry Leaves (e.g., Lutein, β-carotene, Dyes)

Conventional Solvent Extraction method involves soaking the prepared leaf material in a solvent. Water is a common solvent for natural dye extraction (Chetia *et al.*, 2023) [6]. Organic solvents like ethanol are also used for extracting phenolic compounds and flavonoids (Kattil *et al*., 2024) [12]. Optimal ratio of Material to Liquor (M:L) can range (e.g., 1:10, 1:20, 1:30). Elevated temperatures are often used to enhance extraction efficiency (e.g., 60 °C, 70 °C, 80 °C). Extraction time can vary (e.g., 60 minutes, 70 minutes, 80 minutes, 90 minutes). For optimal dye yield from leaves, a material-to-liquor ratio of 1:30, temperature of 80 °C, and extraction time of 80 minutes have been reported. After extraction, the mixture is typically filtered to separate the solid residue from the pigment extract. The liquid extract can then be concentrated (e.g., by drying in a hot water bath to evaporate water) (Chetia *et al*., 2023) [6].

Anthocyanin extraction from Mulberry Fruits

Solvent Extraction is a common laboratory-scale method. Polar solvents are preferred, such as ethanol, methanol, or acetone, often in aqueous solutions (e.g., 70-80% in water) and under acidic conditions (e.g., with 0.1% HCl or trifluoroacetic acid) to enhance recovery of anthocyanins and phenolic compounds (Kattil *et al*., 2024; Qin *et al*., 2010; Suhaimi *et al*., 2021) [12, 16, 18]. Soaking the sample in a large volume of solvent at ambient temperature with occasional shaking. For example, 50% ethanol for 4 hours at 25°C. Ultrasonic-Assisted Extraction method uses ultrasonic waves to improve extraction efficiency. Examples include 63.8% methanol with 1% trifluoroacetic acid at 43.2°C for 40 minutes, or 80% ethanol at 20°C for 12 minutes. Microwave-Assisted Extraction uses microwave energy to heat the solvent and sample, such as 59.6% methanol at 425 W power for 132 seconds (Kattil *et al*., 2024) [12]. In alcoholic extraction, fresh mulberry fruits can be extracted with 95% alcohol/0.1% HCl (1:1 ratio) at room temperature for 4 hours in the dark (Qin *et al*., 2010) [16].

Purification and Concentration

After initial extraction, purification steps are often necessary to increase pigment purity and concentration, especially for anthocyanins. Extracts are typically filtered to remove solid particles (Chetia *et al.*, 2023) [6]. Macroporous resins (e.g., X-5, AB-8) are effective for adsorbing anthocyanins, removing impurities like mono-, di-, and polysaccharides, minerals, proteins, and organic acids. The adsorbed anthocyanins can then be eluted using acidified ethanol (e.g., 0.5% (v/v) hydrochloric acid in ethanol) (Liu *et al.*, 2004; Zhang *et al.*, 2011) [14, 21]. This process can significantly increase the color value (Liu *et al.*, 2004) [14]. The eluted pigment solution can be concentrated using a rotary evaporator under reduced pressure, followed by lyophilization (freeze-drying) to obtain pigment powder (Liu *et al.*, 2004) 14[]. For high-purity isolation of individual anthocyanin monomers, methods like high-speed counter current chromatography combined with column chromatography can be used (Chen *et al.*, 2017) [5]. Sephadex LH-20 columns can be used for separation, eluting with solvents like 10% ethanol containing 1% acetic acid (Zhang *et al.*, 2011) [21].

Analysis and Characterization

After extraction and purification, the pigments are typically analyzed to identify and quantify their components. Techniques like UV spectrophotometry can be used to determine total anthocyanin content using methods like the pH differential method (Liu *et al*., 2004) [14]. HPLC used for identification and quantification of individual pigments and their relative concentrations (Liu *et al*., 2004; Qin *et al*., 2010) [14, 16]. LC-MS and NMR are advanced techniques for detailed identification of pigment structures (Qin *et al*., 2010) [16]. FT-IR used to confirm the presence of specific functional groups like alcohols, halogens, flavonoids, phenols, and tannins in the extract (Chetia *et al*., 2023) [6]. This systematic approach allows for the efficient and targeted extraction of various pigments from different parts of the mulberry plant, catering to diverse applications.

The optimization of pigment extraction from mulberry plants necessitates a detailed evaluation of various methodologies to maximize yield and purity. Pigment extraction from mulberry plants yields a variety of natural coloring agents, primarily from both leaves and fruits. The results of these extractions depend on the specific plant part used, the mulberry cultivar, and the extraction methodology.

Identification of Pigments

Mulberry plants are rich in various pigments and bioactive compounds. From Mulberry Leaves lutein (30.86%) and β-carotene (26.30%) are reported as primary coloring agents (Chetia *et al*., 2023) [6]. Other compounds include quercetin and kaempferol (Chetia *et al*., 2023) [6], as well as flavonoids, phenolic compounds, carbohydrates, halogen group, and tannins (Chetia *et al*., 2023) [6].

Anthocyanins are abundant in mulberry fruits with cyanidin 3-O-rutinoside (60%) and cyanidin 3-O-glucoside (38%) being the most prevalent. Minor anthocyanins like pelargonidin are also present (Qin *et al*., 2010) [16]. Cyanidin-3-glucoside and cyanidin-3-rutinoside are identified as major anthocyanins (Go *et al*., 2022) [9]. The total anthocyanin content varies significantly among cultivars, ranging from 147.68 to 2725.46 mg/L juice, and is influenced by environmental factors such as climate and production area (Liu *et al*., 2004) [14]. Mulberry fruits also contain flavonoids and phenolic compounds (Centhyea *et al*., 2021; Kattil *et al*., 2024) [3, 12].

From Mulberry Leaves (for dye extraction)

Optimal conditions for dye extraction were found to be a material-to-liquor ratio (M:L) of 1:30, a temperature of 80 °C, and an extraction time of 80 minutes. Under these conditions, a dye yield of 13.38±0.59a was achieved. The extracted dye from mulberry leaves produced colors ranging from brown to yellow-brown (Chetia *et al*., 2023) [6].

From Mulberry Fruits (for anthocyanin extraction)

Purification processes, such as using macroporous resins, can significantly enhance the color value of extracted pigments, often increasing it from less than 4.0 to over 100, while also removing impurities and improving stability (Liu *et al*., 2004) [14]. Mulberry pomace, a byproduct of juice production, has shown potential as a source of anthocyanins, with contents exceeding 250 mg/100 g in some varieties, representing 74%-79% of the anthocyanin content in the whole fruit (Zhang *et al*., 2011) [21].

Various extraction methods and their reported yields for anthocyanins from different mulberry fruit types include:

Mulberry Part	Extraction Method	Solvent/Conditions	Anthocyanin Yield	Reference
Black mulberry fruit juice	Maceration	50% ethanol, 25°C, 240 min	137.06 mg/100 g	(Kattil *et al*., 2024) [12] [12]
Red mulberry fruit juice	Maceration	50% ethanol, 25°C, 240 min	126.12 mg/100 g	(Kattil *et al*., 2024)
Black mulberry fruit powder	Microwave-Assisted Extraction	59.6% methanol, 425 W power, 132 s	54.72 mg/100 g	(Kattil *et al*., 2024) [12]
Dried mulberry fruit	Ultrasonic-Assisted Extraction	63.8% methanol + 1% trifluoroacetic acid, 43.2 °C, 40 min	64.70 mg/100 g	(Kattil *et al*., 2024) [12]
Black mulberry fruit juice	Ultrasonic-Assisted Enzymatic Extraction	80% ethanol, 20 °C, 12 min	55.32 mg/100 g	(Kattil *et al*., 2024) [12]
Fresh mulberry fruits	Solvent Extraction	95% alcohol/0.1% HCl (1:1 ratio), room temperature, 4 h	Not specified, but yields abundant anthocyanins (Qin *et al*., 2010) [16]	(Qin *et al*., 2010) [16]

Pigment Characteristics and Extraction Efficiency

Mulberry leaves are a source of natural dyes, primarily containing lutein (30.86%) and β-carotene (26.30%) as main coloring agents. Other compounds such as flavonoids, phenols, and tannins are also present (Chetia *et al*., 2023) [6]. Optimized extraction parameters, including material-to-liquor ratio, temperature, and extraction time, are crucial for maximizing the dye yield from leaves. For instance, a yield of 13.38±0.59% was achieved under specific conditions (1:30 material-to-liquor ratio, 80°C, 80 minutes) (Chetia *et al*., 2023) [6].

Mulberry fruits, conversely, are rich in anthocyanins, which are responsible for their natural color (Kattil *et al*., 2024) [12]. Key anthocyanins identified include cyanidin-3-o-glucoside (c3g) and cyanidin-3-o-rutinoside (c3r), along with pelargonidin-3-glucoside (Kattil *et al*., 2024) [12]. While raw mulberry juice has a low color value due to non-pigment components, purification with resins significantly enhances the color value (to over 100) and improves stability by removing impurities (Liu *et al*., 2004) [14]. This purification process is critical for producing high-quality pigment for commercial use (Liu *et al*., 2004) [14]. The total anthocyanin content in fruits can vary greatly among cultivars, with ranges observed between 147.68 and 2725.46 mg/L juice, and is also influenced by climate and production area (Liu *et al*., 2004) [14].

Applications and Bioactivity

The extracted pigments from mulberry plants offer a wide array of applications due to their coloring properties and bioactive compounds:

- **Natural Dyes:** Mulberry leaves are a promising natural source for dyeing fabrics, offering a potential reduction in the cost of producing natural dyes and promoting the utilization of agricultural byproducts (Chetia *et al*., 2023) [6].
- **Food Colorants:** Mulberry fruit anthocyanins are attractive as natural food colorants. They are water-soluble and provide appealing colors like orange, red, and blue (Liu *et al*., 2004) [14]. The utilization of these anthocyanins as natural food colorants can enhance the overall profitability of the mulberry plant (Liu *et al*., 2004) [14].
- **Health and Therapeutic Benefits:** Beyond their coloring

attributes, mulberry pigments, particularly anthocyanins, possess significant health benefits. They are potent antioxidants and have been reported to improve visual acuity. They also exhibit antineoplastic, radiation-protective, vasotonic, vasoprotective, anti-inflammatory, chemo-protective, and hepato-protective activities (Liu *et al*., 2004) [14] Mulberry is known to protect from liver damage, strengthen joints, facilitate urine release, and decrease blood stress (Kattil *et al*., 2024) [12]. Additionally, other phytochemicals like flavonoids and phenolics in mulberry contribute to properties such as laxative, hypoglycemic, expectorant, anthelmintic, odantalgic, and emetic effects (Kattil *et al*., 2024) [12].

- **Emerging Technologies:** Mulberry pigments also show potential in advanced applications, such as their use as dye sensitizers in Dye-Sensitized Solar Cells (Suhaimi *et al*., 2021) [18].

Conclusion

Mulberry plants, both leaves and fruits, present a valuable and diverse source of natural pigments with broad applications. The successful extraction and purification of these pigments are crucial for their effective utilization in industries ranging from textiles and food to pharmaceuticals and emerging technologies. The increasing demand for natural and sustainable products positions mulberry pigments as a significant area of research and commercial interest, driven by their aesthetic qualities and compelling health benefits. Plant species with varying colors and properties can be harnessed in the textile, printing, cosmetics, and food industries (Alegbe & Uthman, 2024) [1]. The increasing attention toward natural pigments reflects a growing awareness of the ecological impacts and health hazards linked to synthetic colorants (Bakhsh *et al*., 2023; Venil *et al.*, 2020) [3, 20]. Natural pigments are expected to have increased use in the pharmaceutical, medical, and food sectors because of their nontoxic qualities, biodegradability, and biocompatibility (Chetia *et al*., 2023; Masyita *et al*., 2025) [6, 15]. The food industry is turning to natural pigments as consumer awareness grows regarding the possible health risks of artificial food coloring. The use of bacterial pigments shows promise for a wide array of new biotechnological uses, including functional food production Mulberry's rich pigments, particularly anthocyanins, not only serve as natural colorants but also offer various health benefits, including antioxidant, anti-inflammatory, and anti-cancer properties. and the creation of cutting-edge medications and biomedical therapies (Celedón & Barrientos, 2021; Kattil *et al.,* 2024; Go *et al*., 2022) [4, 12, 9]

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